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# WRITING FOR PHYTOPATHOLOGY

John Doe

Professor, Department of Botany, University of Blank.

A report on *Phytopathology* style beginning in 1960.

Note: titlenotes and authornotes are no longer footnotes.
Accepted for publication

SUMMARY

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LITERATURE CITED

1. Price, W. C. 1954. Preparing manuscripts for Phytopathology. Phytopathology 44:667-674.

2. Riker, A. J. 1946. The preparation of manuscripts for Phytopathology. Phytopathology 36:953-977.

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**Table 1.**—Abbreviations to use in text and tables. *Never* use vertical lines in tables.[a]

| Heading | Heading | Heading | Heading |
|---|---|---|---|
| * significant at 5% level | cu cubic | $LD_{50}$ lethal dose, 50% | oz.[b] |
| ** significant at 1% level | $ED_{50}$ effective dose, 50% | log | p.[b] page |
| μ micron | et al | LSD least significant difference | p.m.[b] |
| % per cent | °F | m meter | ppm |
| A.[b] acre | Fig.[b] | m milli- | psi lb/sq. in. |
| Å Angstrom | ft | M[c] molarity | pt |
| a.m.[b] | ft-c | me milli-equivalent | qt |
| amp | g gram | N[c] normality | rep roentgen equivalent physical |
| atm | G gravity | no.[b] | RH relative humidity |
| bu | gal.[b] | ns not significant | rpm |
| °C | in.[b] | W watt | sq |
| cc | kg | | |
| cm | L liter | | |
| | lb | | |

[a]Note 2 general rules: 1) don't add *s* for plurals; 2) superscripts for table footnotes are *letters*.

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[1]Avoid footnotes. They are distracting and costly. Give the information in the text (in parentheses).

# TAKEWO HEMMI
1889–1959

Shigeyasu Akai[1] and G. W. Keitt

Professor Takewo Hemmi passed away on October 12, 1959, at his home in Kyoto after a long illness. Because of ill health he had been on leave of absence from the Osaka Prefectural University, his last post held, since March, 1957.

Takewo Hemmi was born in Takaoka, in northern Japan, on September 15, 1889. Upon graduating in Agriculture from Tohoku University, Sapporo, in 1915, he began postgraduate work in plant pathology under the direction of Professor Kingo Miyabe, founder of plant pathology in Japan, at Hokkaido University, Sapporo, from which he received the doctor's degree in 1920.

His early interest was in the anthracnose diseases. He collected many Japanese specimens and clarified the relation of environmental factors to the mycelial growth and pathogenicity of the causal fungi, and also to the disease outbreak. He published his results in the Journal of the College of Agriculture, Hokkaido Imperial University, in 1920–21.

Prior to his appointment as Professor at Kyoto University, in 1924, he went abroad, and in America he studied the epidemiology of plant diseases at the University of Wisconsin under the direction of Professor L. R. Jones. On his way from America, he traveled in Europe, and after returning home, he was made Director of the Laboratory of Plant Pathology, Kyoto University.

Throughout his career he was interested in the epidemiology of rice plant diseases. He made many outstanding contributions in this field, especially on rice blast. He clarified the physiological characteristics of the pathogenic fungus of the blast disease, *Piricularia oryzae*, and the relation of environmental factors, particularly of rice plant culture, to occurrence of the blast disease. It may be said that he was largely responsible for the basis of the present control measures of the rice blast disease in Japan. His studies also included the silicification of epidermal cell walls in leaves of rice plants in relation to blast resistance and the induced resistance of rice plants against blast disease by treating the seeds or seedlings with chemicals, such as plant growth substances. These contributions were summarized in 1935 in *Phytopathologische Zeitschrift* 6:307-324, and also included in the book, in Japanese, "Studies on blast disease," published by the Asakura Shoten, Tokyo, in 1949.

Diseases of trees and wood-rotting fungi were also included in Dr. Hemmi's interest. He collected many specimens of decayed wood and decay fungi, and in the field of dendropathology are also found many of his accomplished contributions. He was also deeply concerned with market disease. He studied diseases of vegetables and fruits in markets of the Kyoto and Osaka districts, and discussed cause and prevention.

Dr. Hemmi was much interested in the taxonomy of fungi. He made extensive collections of *Septoria*, anthracnose fungi, wood-rotting fungi, and mushrooms. Collections of so-called fleshy fungi, especially Clavariaceae, Pezizaceae, and Geoglossaceae, pleased him very much, and he described some of them.

From 1928 to 1929, and from 1946 to 1950, he was President of the Japanese Phytopathological Society, and was made an Honorary Member of this society in 1952. He was also on the editorial board of *Phytopathologische Zeitschrift* from 1929 and of the *Mycopathologia et Mycologia Applicata* from 1949. In 1931 and 1937, he edited the journal of his laboratory, the Shokubutsu Byôgai Kenkyu (*Forschungen auf dem Gebiet der Pflanzenkrankheiten*), in which he published many articles on experiments conducted there.

After he left Kyoto University, in 1949, he accepted the call as a professor at Osaka Prefectural University, in 1952, where he taught students of plant pathology until he became ill. He was one of the most distinguished scholars of his time and was known as a man of strong conviction and steadfast character.

[1] Professor of Plant Pathology, Kyoto University, Kyoto, Japan.



PHYTOPATHOLOGY for September (50:627-686) was issued September 20, 1960

# THE HELMINTHOSPORIUM GRAMINEUM COMPLEX AND RELATED SPECIES ON CEREALS AND FORAGE GRASSES

Samuel W. Braverman

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SUMMARY

Morphologic, physiologic, and pathologic investigations of *Helminthosporium avenae*, *H. teres*, *H. gramineum*, *H. bromi*, and the *Helminthosporium* stage of *Pleospora phaeocomes* were undertaken to seek criteria for species delineation, specific relationships, and variability within each. Conidia from naturally infected and artificially inoculated hosts were variable in length, whereas conidia produced in culture were smaller and more uniform in size. Conidia of *H. bromi* were significantly larger than those of the other 4 species, both in culture and in nature. *H. avenae* grew better than other species on a culture medium containing malachite green, crystal violet, and 6 or 8% concentrations of ethanol. *H. phaeocomes* was separated from the other species by producing a scant mycelial colony in solid and liquid media containing 8 different N-sources. *H. avenae* grew better with phenylalanine in a liquid medium than the other species, and *H. teres* grew better with potassium nitrate in a solid medium than the other species. *H. gramineum* grew poorly, but significantly better than *H. phaeocomes* on all culture media. Chloroform extracts of dried mycelial colonies of *H. avenae* were red; those of *H. teres*, red-orange; and those of *H. gramineum*, orange. Cultures of *H. bromi* and the asexual stage of *Pleospora phaeocomes* produced no chloroform-extractable visible pigment. Minimum light transmission occurred at wavelengths of 400–500 mμ for *H. teres*, 450–500 mμ for *H. gramineum*, and 500 mμ for *H. avenae*. In cross pathogenicity tests, *H. avenae* infected barley (*Hordeum vulgare*), oats (*Avenae sativa*), tall oatgrass (*Arrhenatherum elatius*), and orchardgrass (*Dactylis glomerata*), whereas *H. teres* and *H. gramineum* infected only barley. *Pleospora phaeocomes* was pathogenic on orchardgrass, and *H. bromi* infected only bromegrass (*Bromus inermis*). It is concluded that the 5 species are distinct.

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In 1951 Luttrell (8) published a key to species of *Helminthosporium* Lk. reported on grasses in the United States. Included was the *Helminthosporium gramineum* Rab. complex, composed of *H. gramineum*, *H. teres* Sacc., and *H. avenae* Eidam, all with more or less cylindric conidia. Criteria for species delineation were conidial size, constriction, color, septation, and host reaction. Christensen and Graham (3) found that *H. gramineum* comprised an indefinite number of races that differed from each other in pathogenicity and in physiological characteristics.

The range in length of conidia of *H. avenae* is so great that measurements at the lower end of the range fit those of *H. gramineum*, and those at the upper limits fit measurements for *H. teres* (7). Drechsler (5) reported that dimensions of conidia of *H. avenae* were equal to those of *H. teres*. Drechsler (5) and Putterill (10), comparing *H. teres* with *H. avenae*, concluded that the longer conidia of *H. teres* are distinct from those of the other species. Drechsler also proposed that the greater numbers of conidiophores in a fascicle be used to distinguish *H. gramineum* from *H. teres*. Confusion exists regarding the morphologic similarity of the 2 species, yet they may be differentiated by their symptom induction on barley (*Hordeum vulgare* L.): *H. teres* produces a net blotch and *H. gramineum* a stripe.

The original description of *H. bromi* Died. (4) rendered this species indistinguishable from *H. teres*. In 1923 Drechsler (5) emphasized the conspicuous difference between the 2 species in conidial length. The conidia of *H. bromi* were significantly longer than those of *H. teres*.

In 1955 Graham (6) described a disease of orchardgrass (*Dactylis glomerata* L.) caused by *Pleospora phaeocomes* (Reb.) Wint. This species possessed a *Helminthosporium* stage with conidial measurements similar to those of *H. gramineum* and was considered to be a closely related species of the complex.

In 1952 Cappellini (1), using cultural characteristics, separated several *Helminthosporium* species, including *H. bromi*, *H. avenae*, *H. teres*, and *H. gramineum*. He proposed a key to species on the basis of differential growth on various culture media.

Nisikado (9) divided the genus *Helminthosporium* into 2 sub-genera, based on morphologic criteria, the *Eu-Helminthosporium* and *Cylindro-Helminthosporium*. Of 40 species of *Helminthosporium* examined biochemically by Raistrick et al (11), 24 had been morphologically compared by Nisikado. All species (with 1 possible exception) that produced polyhydroxyanthraquinones were included by Nisikado in the *Cylindro-Helminthosporium*, whereas the species that produced a polyhydroxyanthone were placed in the *Eu-Helminthosporium*.

In the present investigation, morphologic, physiologic, and pathologic characteristics of isolates of *Helminthosporium avenae*, *H. teres*, *H. gramineum*, *H. bromi*, and the *Helminthosporium* stage of *P. phaeocomes* were studied to seek criteria for species delineation, to delimit variability within each of the species, and to determine specific relationships.

**Materials and methods.**—The isolates used are listed in Table 1. Each monoconidial isolate was stored under sterile mineral oil. Isolates were grown on

**Table 1.**—Number of isolates and origin of various Helminthosporium species used in this investigation.

| Species | No. of isolates | Host | Origin |
|---|---|---|---|
| *H. avenae* | 3 | *Avenae sativa* L. | ATCC, Ga., Pa. |
| | 1 | *Arrhenatherum elatius* (L.) Presl | Pa. |
| *H. bromi* | 3 | *Bromus inermis* Leyss. | ATCC, Pa. |
| *H. gramineum* | 3 | *Hordeum vulgare* L. | Md., Pa. |
| *H. teres* | 4 | *Hordeum vulgare* L. | Canada, Pa. |
| *P. phaeocomes* | 3 | *Dactylis glomerata* L. | Pa. |

Difco potato-dextrose agar and transferred at 3-week intervals. Isolates were cultured at 25±1°C for the growth studies and at 28±1°C for pigment production. Media in each 9-cm Petri dish or 125-ml Erlenmeyer flask were inoculated with a 3-mm disc of mycelium from the edge of a colony. Before autoclaving, media were adjusted to pH 5.5 with 5% KOH or 25% HCl. For pigmentation studies the culture vessels were 1000-ml wide-mouth Erlenmeyer flasks, the inoculum disc was 5 mm, and pH before autoclaving was adjusted to 7.5 with NaOH pellets.

Excised diseased leaves of orchardgrass, bromegrass, tall oatgrass, barley, and oats collected in the field, and leaves of these plants inoculated as seedlings in the greenhouse, were incubated in moist chambers. The morphology of conidia from infected leaves and from spores gathered from a glucose-asparagine medium was studied. For each collection, 25–50 conidia were selected at random and measured to determine extreme and mean dimensions.

The basal medium used in the study of growth-inhibiting substances was 3.0 g Difco malt extract, 2.0 g Difco yeast extract, 0.5 g $KH_2PO_4$, 0.5 g $MgSO_4 \cdot 7H_2O$, and 20.0 g Difco agar in 1000 ml distilled water. Malachite green (1 and 10 ppm) and crystal violet (10 and 20 ppm) were added aseptically as 5-ml portions to 15 ml of medium.

The basal medium used in the study of ethanol as the sole carbon source was composed of 1.0 g $(NH_4)_2SO_4$, 1.0 g $KH_2PO_4$, 0.5 g $MgSO_4 \cdot 7H_2O$, 1 drop of 0.01% Difco yeast extract, and 20.0 g Difco agar in 1000 ml distilled water. Five ml of 6% or 8% ethyl alcohol were added aseptically as separate portions to 20 ml of medium.

The effect of N-containing compounds on the isolates was determined by adding each N-source in the amount necessary to supply 250 mg N per liter. The N-sources were: ammonium acetate, dihydrogen ammonium phosphate, ammonium tartrate, potassium nitrate, glycine, urea, phenylalanine, and asparagine. The basal medium was composed of 3.75 g anhydrous D-glucose, 2.0 g $KH_2PO_4$, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.01 g $FeSO_4$, and 1000 ml distilled water. The medium was solidified with 20.0 g Difco agar purified by the technique of Robbins (12). In all 3 experiments the cultures were harvested after 12 days' incubation.

The basal medium for pigmentation study was 50.0 g anhydrous D-glucose, 2.0 g $KNO_3$, 1.0 g $KH_2PO_4$, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.1 g $FeSO_4$, and 1000 ml distilled water. Each culture vessel held 350 ml of medium. To extract the pigments, mycelium was harvested 6 weeks after inoculation, dried overnight at 85°C, and ground in a mortar. A mixture of 100 ml chloroform and 1 g dried mycelium was extracted for 24 hours in a Soxhlet apparatus. A Coleman spectrophotometer was used to analyze the crude fungus-pigment.

Orchardgrass, bromegrass, tall oatgrass, barley, and oats were grown from seed for pathogenicity tests. Seeds were sown in 4-in. pots of steamed soil and thinned to 3 per pot when the seedlings were 3 in. tall. To assure adequate sporulation, *Helminthosporium* and *P. phaeocomes* isolates were grown on a glucose-asparagine medium similar to that used in the nitrogen studies. Spores and mycelium of each isolate cultured in 6 Petri dishes were added to 200 ml tap water, comminuted for 30 seconds in a Waring blendor, and sprayed on premoistened seedlings with an electric paint sprayer. Three plants each were inoculated in 3 separate tests, covered with a polyethylene bag to create a saturated atmosphere, and placed under a humidifier for 48 hours. The bags were then removed and the plants were left under the humidifier for an additional 48 hours. Disease readings were made 1 week after inoculation.

**Experimental results.**—*Morphology.*—Conidia of *H. bromi* from all sources were significantly larger in mean length and width and had more septations than conidia of the other 4 species (Fig. 1).

Conidia measured from experimentally inoculated hosts were of similar size to those obtained from naturally infected plants. Range in length of conidia was greater for *H. avenae* and *H. teres* than for *H. gramineum* and the asexual stage of *Pleospora phaeo-*

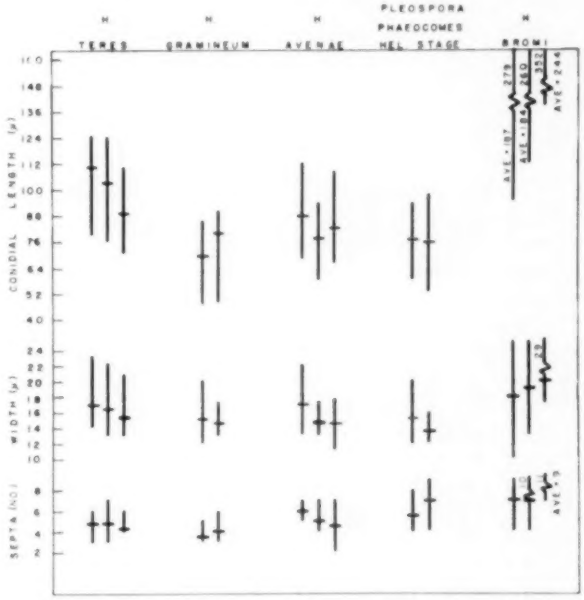


**Fig. 1.**—Measurements and septation of conidia of *Helminthosporium teres*, *H. gramineum*, *H. avenae*, the *Helminthosporium* stage of *Pleospora phaeocomes*, and *H. bromi* from host tissue. Vertical lines indicate the range; horizontal lines, the average.

*comes*. Conidia of *H. teres* were larger than those of the other 3 closely related species. Isolates were cultured on several agar media—corn-meal agar, yeast extract, Sachs, Hopkins, and the various media used in the nitrogen utilization study—to determine which was best for conidial production. Except for *H. gramineum*, all isolates of the 5 species sporulated well on a glucose-asparagine medium. In general, conidia produced in culture retained their characteristic shape but were less variable and shorter than those produced on host tissue.

Except for *H. bromi*, the species could not be differentiated on the basis of conidial width and septation.

*Physiology*.—Physiological characteristics were studied as possible criteria to use in conjunction with the morphological data to delineate the *Helminthosporium* and *Pleospora* isolates. These included reaction to growth-inhibiting substances, ethanol as the sole carbon source, availability of nitrogen sources, and pigment production.

Direct comparisons of the effect of malachite green and crystal violet in a basal malt-yeast agar medium were made at 10 ppm. At this concentration, malachite green had a greater inhibitory effect than crystal violet. Colony diameter at this concentration was greater for *H. avenae* than for the other species. At 10 ppm of crystal violet, colonies of the asexual stage of *P. phaeocomes* were consistently larger than the 3 *Helminthosporium* species from cereals.

*H. avenae* attained significantly larger colony diameters at the 8% concentration of ethanol in a basal medium than isolates of the other species. Minor differences were noticeable, but they were not great enough to distinguish species.

Several of the *Helminthosporium* species grew poorly in liquid media and were therefore grown on a solid medium. Since all species utilized nitrate, ammonium, and organic nitrogen to some extent, they were compared by dry weight of fungus mycelium produced.

The asexual stage of *P. phaeocomes* differed from the closely related species on cereals by its poor growth on all N-containing compounds in both solid and liquid media. *H. avenae* grew best with phenylalanine in a liquid medium, whereas isolates of *H. teres* and *H. gramineum* utilized it poorly. *H. teres* grew best with potassium nitrate in a solid medium, whereas *H. avenae* and *H. gramineum* did not. Variation among isolates of each species was slight.

Chloroform extraction of dried mycelium demonstrated the presence of pigments. These chloroform-soluble pigments were analyzed spectrophotometrically to determine whether species could be differentiated by this method. In general, all isolates of a species were similar in their cultural characteristics on the medium used and produced extracts of similar color. The chloroform-soluble pigments of each species (except for the *Helminthosporium* stage of *P. phaeocomes* and *H. bromi*, which produced no visible pigment) varied in color and possessed different spectral transmittance abilities. The chloroform extracts of 4 isolates of *H. avenae* were red, 4 of *H. teres* were orange-red, and 3 of *H. gramineum* were orange.

The residual pH of the culture filtrate was 6.4–7.4. Variation was greatest in isolates of *H. gramineum*. Since isolates of this species produced extracts of similar color, it is assumed that pigment production is not correlated with any marked alteration of pH.

The per cent light transmittance at wavelength intervals of 25 m$\mu$ was determined for the 3 species that produced visible pigment. Minimum light transmittance of extracts for the 3 species was distinct in the spectrophotometric analysis. All isolates of *H. teres* attained minimum per cent light transmittance at 400–500 m$\mu$. The low points of light transmittance for *H. gramineum* were 450–500 m$\mu$, whereas the low for *H. avenae* was 500 m$\mu$. Extracts of *H. bromi* and the asexual stage of *P. phaeocomes* had transmittance curves identical to that of chloroform, which was used as the control.

*Pathogenicity*.—*H. avenae* parasitized several hosts. On oats, immature lesions appeared as minute streaks that at maturity enlarged into oblong blotches or, in many cases, a general leaf blight. Similar lesions were formed on tall oatgrass. On leaves of barley, immature flecks developed into brown stripes (up to 7 mm long) surrounded by a yellow border. *H. avenae* also infected orchardgrass, producing minute water-soaked spots with light centers. These enlarged into necrotic lesions (4–6 × 2 mm) surrounded by a purplish-brown border.

*H. teres* and *H. gramineum* infected only barley. *H. teres* infection produced a net blotch lesion generally surrounded by a narrow border or blotches and streaks. Infection by *H. gramineum* produced an elongated brown to yellow stripe lesion up to 55 mm long.

The *Helminthosporium* stage of *P. phaeocomes* induced symptoms only on orchardgrass, as previously described by Graham (6). Infection by *H. bromi* incited dark brown streaks (3–6 × 1–2 mm long) surrounded by a yellow border on bromegrass.

**Discussion.**—Not all characteristics employed in mycological identifications are applicable to specific groups. For example, the classification of yeasts is based on physiological reactions. Delimitation of species in the higher groups of ascomycetes has been based primarily on morphological criteria. The usefulness of morphological criteria depends largely on 2 factors—well-defined, genetically determined differences among collections, and a relatively small and measurable effect of the environment on such character expression. When a species is not easily delineated by morphological criteria, supplementary characteristics must be used. These include physiological criteria and host specificity.

Although minor differences were found in length of conidia, with the exception of *H. bromi*, collections of *Helminthosporium* species were closely integrated, and thus species identification based on this criterion could not be justified with any degree of certainty.

Until a study of numerous field collections is undertaken, supplementary criteria such as physiological and pathological studies are needed to substantiate the validity of species differentiation.

Charles et al (2) found that *H. avenae* produced cynodontin, whereas *H. gramineum* produced helminthosporin and hydroxyisohelminthosporin. Since the color extracts and light transmittance curves of all isolates of *H. teres* were different from those produced by *H. avenae* and *H. gramineum* (each species producing different-colored extracts), it may be assumed that *H. teres* contains a mixture of pigments different from the other 2 species.

It is concluded that *H. avenae*, *H. teres*, *H. gramineum*, *H. bromi*, and the *Helminthosporium* stage of *P. phaeocomes* are distinct and should be retained as valid species based on morphological, physiological, and pathological studies.—U. S. Regional Pasture Research Laboratory, University Park, Pennsylvania.

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# HELMINTHOSPORIUM DICTYOIDES AND RELATED SPECIES ON FORAGE GRASSES

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SUMMARY

Morphologic, physiologic, and pathologic studies of several *Helminthosporium dictyoides* isolates from meadow and tall fescue, perennial and annual ryegrass, and from a perennial ryegrass × meadow fescue hybrid (2×PR × 4×MF) revealed 2 physiologic forms of the fungus. The names proposed are *H. dictyoides* f. sp. *dictyoides* n.f. from meadow and tall fescues, and *H. dictyoides* f. sp. *perenne* n.f. from perennial and annual ryegrasses and the hybrid. Conidia of all isolates from the 5 hosts were morphologically inseparable but were distinct from the closely related species *H. dictyoides* var. *phlei*, *H. siccans*, *H. erythrospilum*, and *H. catenarium*. Isolates of *H. dictyoides* from the fescues produced sclerotia-like bodies on potato-dextrose agar, whereas isolates from the ryegrasses and the hybrid did not. The mycelial growth of all isolates was variously inhibited by 2 concentrations of malachite green and crystal violet. Each fungus species grew differently in media containing 8 nitrogen sources. Chloroform extracts of mycelium of *H. dictyoides* harvested from the fescues and *H. dictyoides* var. *phlei* from timothy were beige to yellow, whereas isolates of *H. dictyoides* from the ryegrasses and the hybrid produced no visible pigment. *H. siccans* and *H. erythrospilum* produced a pink pigment, and the extract of *H. catenarium* produced only a trace of color. Isolates of *H. dictyoides* from the fescues were pathogenic only on the fescues, whereas isolates from the ryegrasses and the hybrid were pathogenic on those hosts and to a less degree on the fescues. *H. siccans* was pathogenic on the fescues, ryegrasses, and the hybrid. Cultures of *H. dictyoides* var. *phlei* and *H. erythrospilum* were specific to their respective hosts.

The *Helminthosporium gramineum* Rab. complex on cereals and forage grasses was discussed in another paper (1). Species delineations in that complex were based on morphologic, physiologic, and pathologic criteria. In the present study these criteria were applied to another group of *Helminthosporium* species on forage grasses to determine a basis for taxonomic separation.

Leaf lesions caused by a species of *Helminthosporium* Lk. were found on plants of a triploid hybrid

**Table 1.**—Number of isolates and origin of various *Helminthosporium* species used in this investigation.

| Species | No. of isolates | Host | Origin |
|---|---|---|---|
| *H. catenarium* | 3 | *Cinna arundinacea* L. (Cinnagrass) | Pa., Canada |
| | 1 | *Agrostis tenuis* Sibth. (Bentgrass) | Pa. |
| *H. dictyoides* | 9 | *Festuca elatior* L. (Meadow fescue) | Pa., N.C., N.Y., W. Va. |
| | 4 | *Festuca arundinacea* Schreb. (tall fescue) | Pa., N.Y., W. Va. |
| | 3 | *Lolium perenne* L. × *F. elatior* L. (hybrid) | Pa. |
| | 4 | *Lolium multiflorum* Lam. (annual ryegrass) | Pa., N.Y. |
| | 5 | *Lolium perenne* L. (perennial ryegrass) | Pa., N.Y., W. Va. |
| *H. dictyoides* var. *phlei* | 5 | *Phleum pratense* L. (timothy) | Pa., N.Y. |
| | 1 | Timothy seed | Scotland |
| *H. erythrospilum* | 2 | *Agrostis alba* L. (redtop) | Pa., Md. |
| *H. siccans* | 3 | *Lolium perenne* L. (perennial ryegrass) | Centraal Bureau voor Schimmelcultures, Md., W. Va. |

[2× perennial ryegrass (*Lolium perenne* L.) × 4× meadow fescue (*Festuca elatior* L.)] developed at the U. S. Regional Pasture Research Laboratory. Diseased leaves showed irregular, brown blotches consisting of short, indistinct horizontal and longitudinal streaks or more often a solid brown band up to 1 in. long extending across the blade.

In 1923, Drechsler (4) described a new species of *Helminthosporium* (*H. dictyoides* Drechs.) that induced a net-blotch on leaves of meadow fescue. It has been found to be common on tall fescue (*F. arundinacea* Schreb.) in the United States. In the same paper he also identified a closely related species (*H. siccans* Drechs.) that induced tiny, dark-brown, elongated, longitudinal spots and a leaf blight on the ryegrasses. Conidia of the latter species were darker and less tapering than those of *H. dictyoides*. No inoculation studies were reported in this work. In England *H. dictyoides* is believed to be the cause of a net-blotch on *Lolium* sp. (3).

In Wales perennial ryegrass is reported to be the principal host of *H. siccans* (9), but the annual species (*L. multiflorum* Lam.) and meadow fescue also are attacked. Dovaston (4), in Scotland, described a new species of *Pyrenophora* (*P. lolii* Dovaston) from a pure culture of an isolation from annual ryegrass. The species induced netlike lesions on annual ryegrass. Conidia were reportedly similar to those of *H. siccans*, but the asexual stage was not described.

*H. dictyoides* var. *phlei* Graham causes a leaf streak of timothy (7) and is not known to attack meadow and tall fescue and perennial and annual ryegrass. *H. catenarium* Drechs. (5) and *H. erythrospilum* Drechs. (6) produce conidia somewhat similar to those of *H. dictyoides*, but these species have received little attention since they were originally described, respectively, on *Cinna arundinacea* L. and *Agrostis* spp.

Criteria for delineation of species and forms were sought by comparing the morphology, physiology, and pathogenicity of *Helminthosporium* isolates from meadow and tall fescues, perennial and annual ryegrasses, the hybrid, and known isolates of *H. dictyoides* and *H. siccans*. Three closely related fungi, *H. dictyoides* var. *phlei*, *H. catenarium*, and *H. erythrospilum*, were also included.

Methods used in this investigation have been described (1). The isolates used are listed in Table 1.

**Morphology.**—*Host plant.*—The morphology of the fungi was studied on infected leaves collected in the field and placed in moist chambers, on plants inoculated in the greenhouse, and on agar media. Two to 4 field collections of infected leaves from the 9 hosts were made at various times during the growing season to study morphology on the host. Also, conidia were measured from plants inoculated with spores from host tissue and from cultures. These represented collections from 1–4 locations. For comparison, leaves inoculated with known isolates of *H. dictyoides*, *H. siccans*, *H. catenarium*, and *H. erythrospilum* were treated similarly. Morphology was studied of fruiting structures from all plants infected in cross-inoculation tests as well as from the original hosts.

Conidia of the *H. dictyoides*-type from meadow and tall fescue, annual and perennial ryegrass, and the hybrid were morphologically similar to known isolates of *H. dictyoides* and agreed with Drechsler's description of the species (Fig. 1). Spores are light green to light brown and taper slightly to sharply toward the apex. The conspicuous hilum is enclosed by the exospore. The basal cell is either hemispherical or hemi-elliposoidal, and the basal or second cell is usually the largest segment of the spore (Fig. 2). A few secondary conidia were found in one collection of *Helminthosporium* from the hybrid. Conidia of *H. dictyoides* var. *phlei* also taper but are predominantly catenulate and smaller than spores of *H. dictyoides*. *H. catenarium* has tapered, nearly colorless, catenulate conidia that are significantly larger than any spores of the *H. dictyoides* complex.

Since *H. siccans* is found on perennial ryegrass, the fungus and symptoms it causes are often confused with those induced by *H. dictyoides* on that host. The olive to brown conidia are cylindrical, or taper slightly toward either end, and are borne on long conidiophores (up to 227 $\mu$, compared with 123 $\mu$ for *H. dictyoides*). Conidia of *H. erythrospilum* on redtop are nearly cylindrical, are borne on long conidiophores, and were the shortest of the species studied.

Considered separately from other morphological characteristics, conidial width and septation could

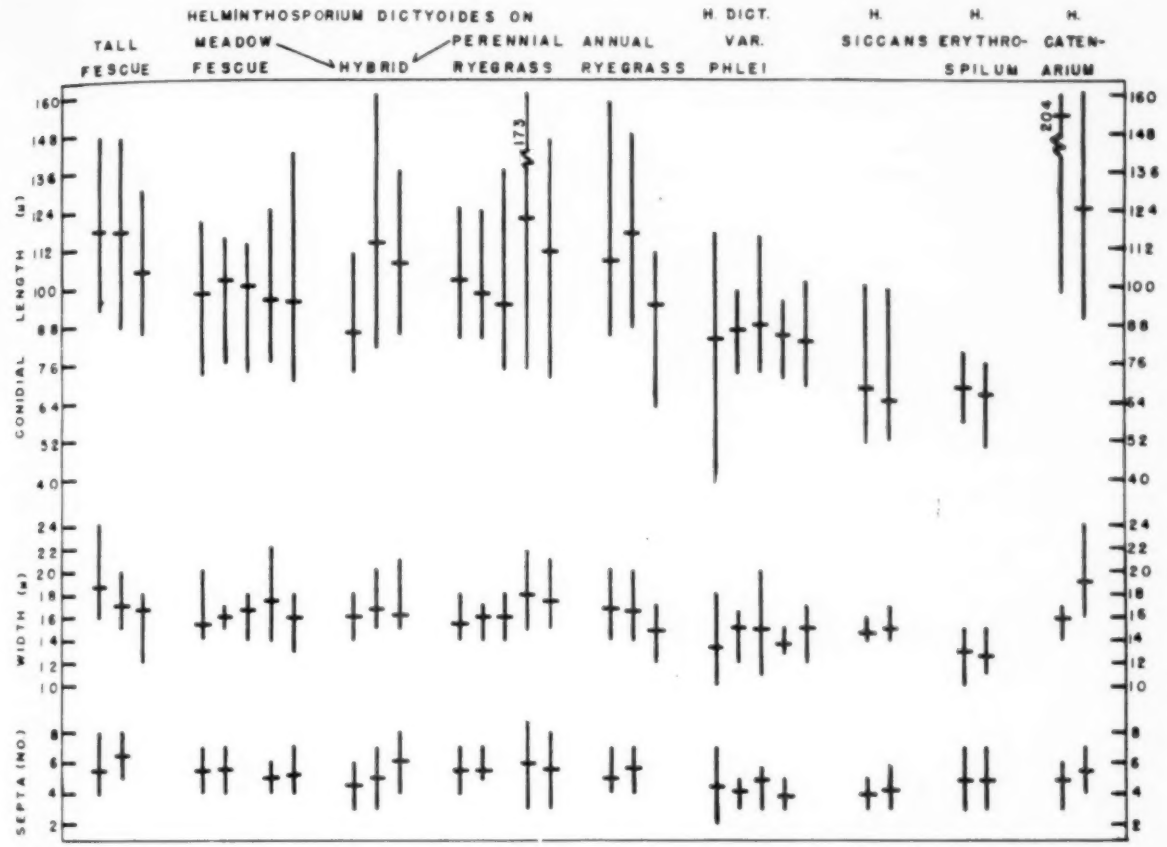


**Fig. 1.**—Morphology of conidia of Helminthosporium on host tissue. Vertical lines indicate the range of one collection; horizontal lines are average.

not be used as criteria for species delineation. In many cases conidia of *H. dictyoides* varied significantly on different hosts; conidia were not consistently larger or smaller on the original host than on an inoculated host, however.

*In culture.*—Isolates were cultured on various media to determine the best for conidial production. These included the following agar media: Corn-meal, yeast-extract, Hopkins, Sachs, and various media used in the nitrogen-utilization study. All isolates except *H. catenarium* sporulated well on glucose-asparagine agar. Conidia retained their characteristic shape on agar media, but were less variable and shorter than on host tissue.

Species and isolates of the fungi could not be distinguished by colony characteristics; however, *H. siccans*, *H. erythrospilum*, and *H. dictyoides* from the fescues produced many sclerotialike bodies in the potato-dextrose agar medium, whereas *H. catenarium*, *H. dictyoides* var. *phlei* and *H. dictyoides* from the

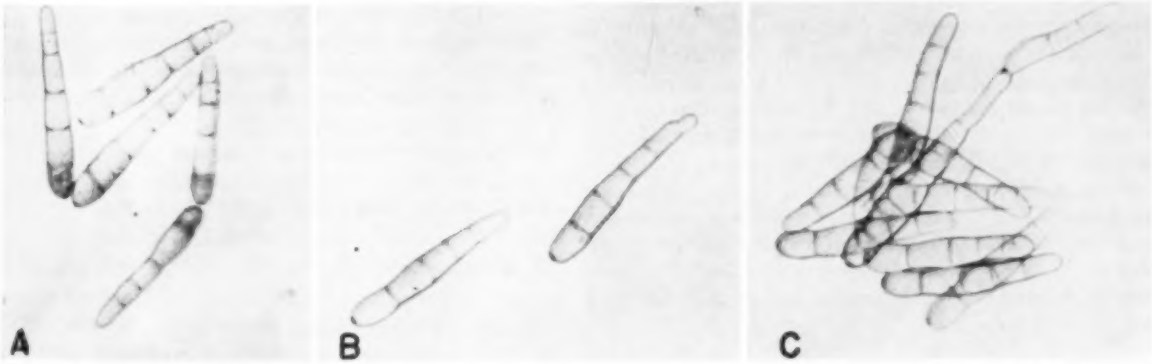


**Fig. 2.**—Conidia of *Helminthosporium dictyoides* from **A)** meadow fescue, **B)** perennial ryegrass, and **C)** perennial ryegrass-meadow fescue hybrid.

**Table 2.** Reaction of 6 forage grasses to isolates and species of *Helminthosporium*.

| Species and source of isolates | Disease index[a] on leaves of indicated grasses | | | | | |
|---|---|---|---|---|---|---|
| | Tall fescue | Meadow fescue | Perennial ryegrass-meadow fescue hybrid | Perennial ryegrass | Annual ryegrass | Timothy |
| *H. dictyoides*-type | | | | | | |
| from tall fescue | 7 | 6 | 0 | 0 | 0 | 0 |
| meadow fescue | 7 | 7 | 0 | 0 | 0 | 0 |
| hybrid | 1 | 3 | 7 | 5 | 5 | 0 |
| perennial ryegrass | 2 | 2 | 4 | 7 | 4 | 0 |
| annual ryegrass | 1 | 2 | 3 | 6 | 5 | 0 |
| var. *phlei* | 0 | 0 | 0 | 0 | 0 | 8 |
| *H. siccans* | | | | | | |
| from perennial ryegrass | 4 | 3 | 2 | 5 | 4 | 0 |

[a] Disease index: 0 = no symptoms; 8 = 80% of blade discolored or killed.

ryegrasses and the hybrid did not.

**Physiology.** — Physiological characteristics were studied as possible criteria to use in conjunction with morphological data to delineate the *Helminthosporium* isolates. These included reaction to growth-inhibiting substances, availability of nitrogen sources, and pigment production.

*Reaction to growth-inhibiting substances.*—Malachite green at 1 and 10 ppm and crystal violet at 10 and 20 ppm were added aseptically to a basal malt-yeast agar medium. Preliminary experiments had indicated that growth differences among isolates of *Helminthosporium* were greatest at these concentrations. The mycelial growth of all isolates was greatly inhibited at higher concentrations of both dyes and slightly to greatly inhibited at the lower concentrations. The variation among isolates was almost as great as that between species.

*Availability of nitrogen sources.*—Growth of the 4 species and the variety of *Helminthosporium* (2–5 isolates of each) from 9 hosts was observed in a basal medium containing the following sources of nitrogen added singly: ammonium acetate, dihydrogen ammonium phosphate, ammonium tartrate, potassium nitrate, glycine, urea, phenylalanine, and asparagine.

General conclusions were drawn from the data even though variation among isolates of each form or species of *Helminthosporium* was slight because each fungus species responded differently to 1 or more of the nitrogen sources. There was no correlation among the 3 methods of growth determination, i.e., weighing the mycelium produced, and on solid media by measuring the diameter of a colony and then weighing the mycelium. In liquid media all species except *H. siccans* produced the most growth in the asparagine medium, whereas on solid media ammonium acetate was the best source of nitrogen for all fungi by weight except *H. catenarium*. Diameters of colonies on solid media differed for each fungus isolate and for each source of nitrogen.

*Pigment production.*—As indicated in an earlier paper (1), pigment production by *Helminthosporium* spp. has been observed and studied by several workers. Charles et al (2) and Raistrick et al (8) purified and identified some of the pigments. Of the species used in this investigation, only *H. catenarium* was studied by the English workers. *H. catenarium* produced catenarin, a hydroxyanthraquinone. Analysis of pigments from other species of the genus suggested differences.

Chloroform extracts containing pigments from dried colonies of the 4 *Helminthosporium* species varied in color and color intensity, and produced different spectral transmittance curves. Isolates of the same species except in the *H. dictyoides* complex produced similarly colored extracts. The extracts of 3 isolates of *H. siccans* and of 2 isolates of *H. erythrospilum* were pink; 3 isolates of *H. catenarium* produced only a trace of color. Chloroform extracts from 4 isolates of *H. dictyoides* var. *phlei*, 3 isolates of *H. dictyoides* from meadow fescue, and 3 from tall fescue were beige to yellow. Colorless extracts were obtained from 3 isolates of *H. dictyoides* respectively from perennial ryegrass, annual ryegrass, and the perennial ryegrass-meadow fescue hybrid.

The original pH of the Czapek-Dox culture medium, 7.5, dropped to 7.0–6.1. Greatest variation in residual pH was among isolates of *H. dictyoides* var. *phlei*. Since extracts from this fungus were similar in color despite variability in pH value, it seems that color differences were not due to differences in residual pH. Minimum and maximum per cent spectral transmittance of the pigment extracts were similar for 2-4 collections of each organism.

In spectrophotometric analyses, minimum spectral transmittance was distinct for each species that produced a colored extract. The per cent light transmittance was determined at 25-m$\mu$ intervals. The low for *H. erythrospilum* and *H. siccans* was 525 m$\mu$; for *H. dictyoides* var. *phlei* and *H. dictyoides* from meadow fescue, 500 m$\mu$; and for *H. dictyoides* from tall fescue, 400 m$\mu$ (the lowest m$\mu$ measured). Isolates within a fungus species (or variety) varied in per cent spectral transmittance, indicating differences in quantities of pigment or pigments.

*Pathogenicity.*—Numerous cross-inoculations were conducted over a 4-year period in the greenhouse to determine the reaction of meadow and tall fescues, annual and perennial ryegrasses, the hybrid, and timothy to known isolates of *H. dictyoides* and *H. siccans* and to several isolates of *Helminthosporium* collected from these hosts at different locations. Preparation of inoculum was described earlier (1).

The average disease ratings for 15 tests are presented in Table 2. *Helminthosporium* from tall and meadow fescue attacked only those hosts, and induced net-blotch on meadow fescue leaves and a net-blotch or long necrotic streaks on tall fescue. *H. dictyoides*-type isolates from the ryegrasses and the ryegrass-fescue hybrid infected those hosts and were less pathogenic to the fescues. Small brown streaks and yellow-brown blotches developed in varying degrees on all hosts. *H. siccans* attacked the same plants, producing necrotic spots with chlorotic margins. Isolates of *H. dictyoides* var. *phlei* from timothy infected only timothy.

In limited inoculation studies, *H. erythrospilim* produced straw-colored spots with dark borders on redtop. *H. catenarium* failed to attack any of the plants used.

**Discussion.**—Conidia harvested from the fescues, ryegrasses, and the hybrid were morphologically similar. Variations in conidial length were apparent, but such variations also occurred within collections from the same host. Little variation occurred in width or septation. Culturally, all isolates exhibited considerable variation, but only isolates from meadow and tall fescue produced sclerotialike bodies in culture. Because all isolates differed in reaction to dyes and availability of nitrogen sources, no groupings could be made on these bases. The isolates were separated into 2 groups on the basis of pigment production. All isolates from the fescues produced a beige to yellow pigment or pigments that differed when analyzed by spectrophotometry. Isolates from the ryegrasses and the hybrid produced no visible pigment. Isolates from meadow and tall fescue infected only those hosts, whereas isolates from annual and perennial ryegrass and the hybrid attacked those hosts and, to a lesser degree, the fescues. From similar responses and morphologic characteristics, it is concluded that *H. siccans*, *H. erythrospilum*, *H. catenarium*, and *H. dictyoides* should be retained as valid species of *Helminthosporium*, and *H. dictyoides* var. *phlei* as a valid variety.

On the basis of the morphological similarity and the physiological and pathological differences, it is therefore proposed that the *Helminthosporium* from meadow and tall fescue and the *Helminthosporium* from annual ryegrass, perennial ryegrass, and the perennial ryegrass-meadow fescue hybrid be designated as formae speciales of *H. dictyoides*.

**Helminthosporium dictyoides** Drechs. f. **dictyoides,** f. nova

Descriptio specifica amendata: corporibus sclerotialibus in culturis productis; maculas interdum reticulatas in foliis *Festuca elatiori* et *F. arundinaceae* efformans.

**Helminthosporium dictyoides** Drechs. f. **perenne,** f. nova

Corporibus sclerotialibus in culturis carentibus; maculas in foliis *Lolii perennis*, *L. multiflori* et hibridae *L. perennis* × *F. elatoris* efformans; etiam in *Festuca elatiori* et *F. arundinacea* artificiose inoculata parce noxia.

**Helminthosporium dictyoides** f. **dictyoides,** forma nova

Amend original description. Sclerotial bodies produced in culture; causing a net-blotch and blotch on leaves of *Festuca elatior* and *F. arundinacea*.

**Helminthosporium dictyoides** f. **perenne,** forma nova

No sclerotial bodies produced in culture; blotch on leaves of *Lolium perenne*, *L. multiflorum*, and *L. perenne* × *F. elatior* (2×PR×4×MF) hybrid. In artificial inoculations, slightly pathogenic on *Festuca elatior* and *F. arundinacea*.—U. S. Regional Pasture Research Laboratory, University Park, Pennsylvania.

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# BACTERIAL STEM ROT OF DIEFFENBACHIA

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SUMMARY

A serious disease of *Dieffenbachia picta*, *D. picta* 'Rudolph Roers,' *D.* × *bausei*, and, to a lesser extent, *D. amoena* has occurred in greenhouses near Los Angeles. Affected plants wilt, followed rapidly by the appearance of typical soft rot symptoms occurring near the soil or at cutting wounds. In 2–3 weeks the stem rots completely and the plant collapses. Internally, light to dark brown streaks occur, progressing inwardly and vertically. Cuttings and cane often rot during propagation. Bacteria occur in abundance within vessels, xylem parenchyma, and resin duct canals. The bacteria move rapidly through these tissues from initial infection sites to other portions of the plant. Pathological and bacteriological determinations indicate that the bacterium from Dieffenbachia is a strain of *Erwinia chrysanthemi*. The disease is controlled by combining protective, eradicative, and sanitary procedures. Partial control may be obtained by dipping cuttings in Agri-mycin 100 (Streptomycin and Terramycin) and propagating them in a thoroughly disinfested greenhouse. Remaining bacteria may be eradicated by treating cane 1.0–1.5 in. in diameter 30–60 minutes in water held at 120°F, and propagating the treated cane in a place well separated from old, contaminated plantings. A correlation was made of the thermal death point of the bacterium and heat penetration of cane, which is a function of stem diameter.

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A serious disease of Dieffenbachia occurred in March, 1956, in greenhouses near Los Angeles. Newly rooted cuttings and stock plants of *Dieffenbachia picta* (Lodd.) Schott, *D. picta* (Lodd.) Schott 'Rudolph Roers,' and *Dieffenbachia* × *bausei* (*D. picta* × *D. weirii*) were affected with a brown, watery rot at the base of the stems. *D. amoena* Hort. ex Gentil was not as severely affected. Large numbers of bacteria were observed in the infected areas. Since the disease had not been reported previously this study was initiated.

**Symptoms.**—*External.*—The earliest external symptom of infection is a wilting of the lower leaves. With artificial infections, wilt occurs 5–7 days after inoculation of the stems. Wilting is followed by darkening and water soaking of the stem at or below the soil line. As the dark areas enlarge the epidermis ruptures, exposing dark-brown, slimy, rotten tissues (Fig. 1-A). Eventually the stem rots completely and the plant collapses. Occasionally a bud below the rotted areas produces a new shoot, which may grow apparently unaffected for several months, but eventually it, too, succumbs. When the stems are inoculated by the needle-puncture method, blisters 0.5–0.75 in. in diameter occasionally form at the site of inoculation. The watery content of the blisters is clear and colorless and contains few bacteria.

Affected areas arising from natural infections progress inwardly from the point of entrance on the stem, whereas those resulting from needle punctures progress uniformly throughout the stem. Cuttings, infected during rooting, are usually rotted from the base upward, and eventually all the stem tissues show soft rot. Canes often develop a typical soft rot during propagation.

*Internal.*—In natural infections light- to dark-brown streaks occur, progressing inwardly and vertically. Infected tissues are firm initially, but eventually they become soft and rotten (Fig. 1-B). Bacteria are abundant throughout the rotted areas. In the solid light-brown areas before the tissues became disorganized, the bacteria are located within xylem vessels, parenchyma, and, in particular, the resin duct canals. In 3 days, the bacteria move more than 20 cm from the point of inoculation in petioles. These observations confirm the vascular spread of the disease, which is similar to that reported for *Erwinia chrysanthemi* Burkholder et al on carnation, *Dianthus caryophyllus* Linn. (8), and *Chrysanthemum morifolium* Ram (5).

**The pathogen.**—Bacteria were easily isolated from infected stems. Other species of bacteria, however, were constantly associated with the pathogen. Results were best with the following technic. A portion of infected stems at the junction of the healthy and diseased areas was selected, the heavily infected tissue trimmed off, and the remaining piece thoroughly washed with hand soap and water. After being blotted on a clean towel the piece was soaked 2 minutes in 0.5% calcium hypochlorite, drained, and trimmed with a sterile scalpel into pieces less than 0.25 in. on a side. The pieces were steeped 10 minutes in 10 ml sterile water to allow the bacteria to emerge. Several dilutions from the infusion were prepared (1:10, 1:100, 1:1000) and 1 drop added per Petri dish, and swirled into approximately 15 ml cooled yeast dextrose calcium carbonate agar (YDC). Usually 1 dilution was suitable for isolating bacteria from single colonies. Such isolated colonies were frequently contaminated with *Pseudomonas* spp., and the pathogenic bacteria were frequently overgrown by the saprophytes. At the suggestion of Professor M. P. Starr this difficulty was overcome by transferring single colonies to 5 ml sterile water containing about 0.01 g of a detergent (Lakeseal Laboratory Glass Cleaner, manufactured by Finger Lakes Chemical Co., Etna, N. Y.) and shaking for 2 hours. Then the bacteria were streaked on YDC plates and incubated 48 hours, and single colonies were transferred to slants. This method reduced the numbers of bacteria somewhat but purified the cultures successfully. The virulence of isolates and their stability in culture were greatly increased by this technic.

Growth was abundant on YDC, and sparse on beef peptone agar (BPA) and potato-dextrose agar (PDA). The following colony descriptions apply to growth on YDC. Colonies appeared in 48 hours and were well

developed in 3–5 days at 20–25°C. Well isolated colonies were creamy-white, raised, with smooth margins, and 0.5–1.0 mm in diameter. The colonies remained discrete and did not spread over the surface of the medium. As colonies aged, the surfaces were frequently beaded or wrinkled when viewed with a dissecting microscope. Small exudates of amber liquid appeared on the surface of the beaded colonies. The surface of the wrinkled colonies appeared shriveled and somewhat dry. This appearance was most common on the large isolated colonies, and not evident on crowded colonies. The colony character was not stable, since each type produced colonies of the other type when subcultured.

Purified isolates were characterized bacteriologically in comparisons with authentic stock cultures (by Dr. D. D. Sutton in 1957, and Mrs. Gladys Cosens in 1959, under Professor Starr's direction in the Department of Bacteriology, University of California, Davis). The isolates, made 2 years apart from naturally infected plants, were of proved pathogenicity and purity before being subjected to bacteriological tests. Dr. Sutton considered the bacterium to be closely allied, but not identical to *E. chrysanthemi*. Mrs. Cosen agreed closely with Dr. Sutton, finding that the Dieffenbachia isolate had much in common with the "Parthenium," "Chrysanthemum," and "Dianthus" isolates of Erwinia as outlined by Hellmers (7, p. 124), although differing from each.

If Hellmer's (7) classification of various Erwinias found on guayule, *Parthenium argentatum* Gray (*Erwinia carotovora* f. sp. *parthenii* Starr), carnation (*E. chrysanthemi* sensu Lelliott), and chrysanthemum (*E. chrysanthemi* Burkholder et al) into forms of *Pectobacterium parthenii* (Starr) Hellmers, is followed, it appears likely that the Dieffenbachia isolate would be named *P. parthenii* var. *dieffenbachii*. However, the combining of various Erwinias into a new genus, Pectobacterium, has not been universally accepted (3, 4, 6, 8, 9). Burkholder (4) recently argued strongly against such a classification, and it appears that the use of Pectobacterium for these groups is premature. It seems best at present to follow the precedent set by Lelliott (8) and withhold naming the bacterium until our comprehensive studies of the group are completed. Consequently, the Dieffenbachia pathogen is considered herein as a strain of *Erwinia chrysanthemi*.

**Inoculations and fulfillment of Koch's postulates.**—Seven inoculation experiments used single-colony isolates of bacteria, and numerous inoculations used infected tissue macerates. In all cases typical symptoms of the disease were reproduced. First symptoms appeared 1–3 weeks after inoculation, and death occurred in 2–6 weeks. Disease progression was fastest in summer and slowest in winter. In 1957 and 1959 bacteria were re-isolated from inoculated plants identified on both occasions as outlined previously, and used to reproduce the original symptoms.

Since several other species of bacteria were so ubiquitously associated in infected hosts with the Erwinia, the possible parasitic role of the associated bacteria was investigated. Four isolates from Dieffenbachia were used, the presently described Erwinia, *Pseudomonas* sp., and 2 unidentified species. Two *D. picta*, 1 *D. picta* 'Rudolph Roers,' and 1 *D. picta* × *bausei* were inoculated with each isolate singly or in

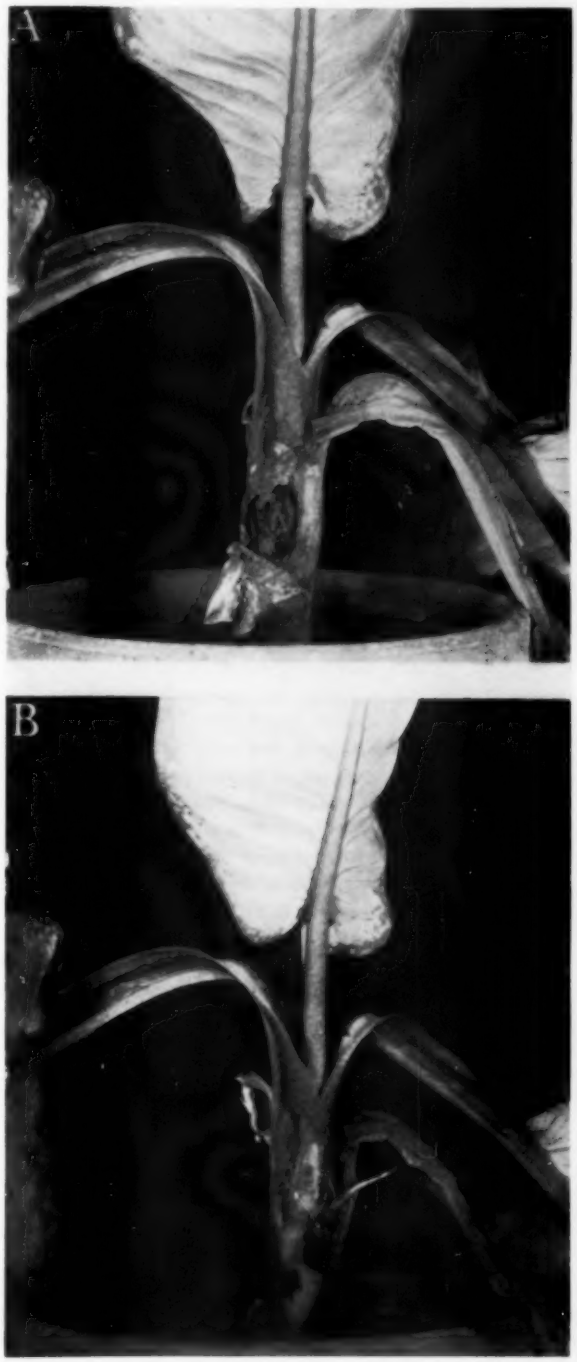


**Fig. 1.**—**A)** *Dieffenbachia picta* 'Rudolph Roers' showing external appearance of stem infection with the Dieffenbachia strain of *Erwinia chrysanthemi*. **B)** Same as A, showing internal appearance of the stem infection.

all combinations of isolates. All of the 32 plants inoculated with the Erwinia were killed, all of the other plants were symptomless. No synergistic or antagonistic effects were noted. Thus, the associated bacteria were strictly saprophytic.

**Host range.**—Limited attempts were made to investigate the host range of the pathogen. The following hosts of *E. chrysanthemi* were tested: *Chrysanthemum morifolium* Ram (varieties Grand Slam, J. W. Prince, Forty-niner, Mary MacArthur, White Top), and *Dianthus caryophyllus* Linn. (White Sim, Pink Sim, Peppermint). Also inoculated were *Parthenium argentatum* Gray, *Lilium longiflorum* Thun., *Philodendron hastatum* (see 2 for nomenclature), *Lycopersicum esculentum* Mill (variety Pearson), and *Pelargonium hortorum* Bailey (var. Radio Red). Stem puncture inoculations were made with pure cultures of the bacteria. No wilt resulted with chrysanthemum inoculated and held 48 hours at 80–90°F and 70–100% RH, the conditions reported as most favorable for disease development caused by *E. chrysanthemi*. The pith of some plants was somewhat darkened after 2–3 months. This reaction was similar to the reported reaction of *E. chrysanthemi* at low temperature and low RH (5). No symptoms were produced on carnation, the other host of *E. chrysanthemi* used in these tests.

Lily scales, contained in Petri dishes and inoculated by swabbing bacteria over fresh needle punctures, remained unaffected. No external symptoms were produced on *P. hastatum*, although the center of the stems at the point of inoculation was rotted slightly after 3 months. There was no spread from the inoculated area. No response could be observed on guayule or geranium.

The reaction of tomato was unusual. Within 7–14 days of stem inoculation, that leaf petiole above the point of inoculation wilted and became necrotic on the underside at the stem junction. In the following 2 months the wilt and necrosis progressed upward. The infected tissues remained brown and dry, and soft rot never developed. The dry rot progressing upward was quite different from the other host reactions. The vascular tissues were discolored. In a plant kept 5 months, new shoots were produced from the stem below the inoculation point.

*Daucus carota* Linn., *Solanum tuberosum* Linn., *Raphanus sativus* Linn., and *Cucumis sativus* Linn. slices were inoculated by swabbing cultures of the bacterium over their surfaces and incubating in the dark at 27°C. Rotting occurred in 18 hours. The cell walls apparently were dissolved since the tissues became water-soaked or, as in radish, the color from the outer tissues diffused throughout the interior of the slice. Epidermal tissues of the slices remained intact. The odor produced was pungent and somewhat like ammonia. No sulfur odors were detected until after 24 hours. In 8 days the slices were completely rotted but still retained their shape.

**Control.**—*Antibiotic studies.*—The sensitivity of the bacteria to streptomycin sulfate, oxytetracycline, and Agri-mycin 100 (streptomycin, 15%, oxytetracycline, 1.5%), and Acti-dione (cycloheximide) was determined *in vitro* by the filter-paper assay-disc technic, using YDC as the medium. The bacteria were more sensitive to oxytetracycline than to streptomycin, and the combination of the two in Agri-mycin 100 was additive but not synergistic. The bacteria were unaffected by doses of Acti-dione up to 100 ppm.

The sensitivity of the bacteria to Agri-mycin 100 was utilized to obtain commercial control of the disease on Dieffenbachia. By dipping cuttings for 15 minutes in Agri-mycin 100 solution (200 ppm active ingredients) before rooting, the cutting rot phase of the disease was reduced to negligible proportions in the first month. The infection in stock plants growing in beds or in large pots was not controlled with Agri-mycin 100, even though weekly sprays (200 ppm active ingredients) were applied for several months.

Since the antibiotic dip and spray control measures were merely palliative, measures involving heat therapy were investigated to obtain an eradicative treatment.

*Hot-water therapy.*—1) *Thermal death point of the bacterium in vitro.*—Forty-eight-hour BPB cultures of the Dieffenbachia pathogen were used either at full strength or diluted 1:10 in sterile distilled water. Tubes containing 5 ml of the cultures were immersed in water held at 110–140°F in increments of 5°F for 10, 20, or 40 minutes. The thermal death point was 120°F/10 minutes for the concentrated broth cultures, and somewhat lower for the diluted series. Some of the saprophytic bacteria survived 140°F/40 minutes. This again demonstrates the competitive ability of these saprophytes.

2) *Effect of hot water on host.*—The thermal death point of the Dieffenbachia pathogen is close to the temperature at which cane is killed. It has been shown (1) that Dieffenbachia cane withstands water temperatures of 120°F, but sometimes succumbs to 125°F.

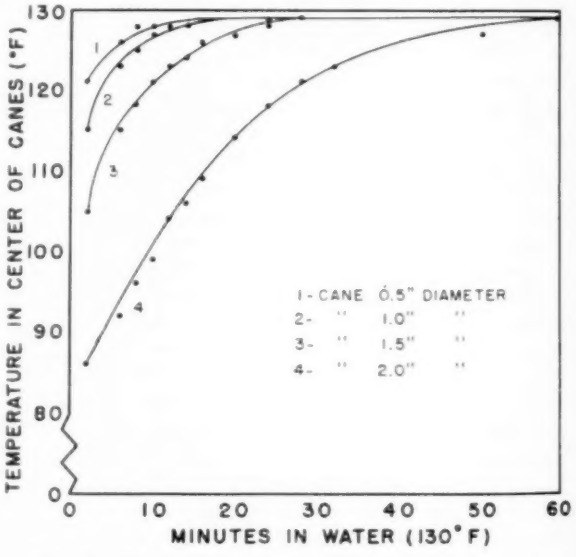


**Fig. 2.**—Effect of size of *Dieffenbachia picta* cane on heat penetration.

**Table 1.**—Rooting of the cane and survival of bacteria in bacterial-infected and uninfected cane of Dieffenbachia picta 'Rudolph Roers' treated with hot water.

| Treatment | Number of treated cane pieces producing roots when propagated: Plant number 1 | 2 | 3 | 4 | 5 | 6 | % surviving | Number of plants infected when inoculated with infusion from hot-water-treated cane: Plant number 1 | 2 | 3 | 4 | 5 | 6 | % infected |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Uninfected series: | | | | | | | | | | | | | | |
| 80°F/60 min. | 1/2 | 1/3 | 1/3 | — | — | — | 37 | 0/1 | 0/1 | 0/1 | 0/1 | 0/1 | 0/1 | 0 |
| 120°F/15 min. | 3/3 | 3/3 | 3/3 | — | — | — | 100 | | | Not used | | | | |
| 120°F/30 min. | 0/2 | 3/3 | 2/2 | — | — | — | 71 | | | Not used | | | | |
| 120°F/60 min. | 0/2 | 0/2 | 0/2 | 1/3 | — | — | 11 | | | Not used | | | | |
| Infected series: | | | | | | | | | | | | | | |
| 80°F/60 min. | 0/2 | 0/2 | 0/3 | 0/2 | 0/2 | 0/2 | 0 | 1/1 | 1/1 | 1/1 | 1/1 | 1/1 | 1/1 | 100 |
| 120°F/15 min. | 0/2 | 0/2 | 0/2 | 0/3 | 0/3 | 0/3 | 0 | 1/1 | 1/1 | 1/1 | 1/1 | 1/1 | 1/1 | 100 |
| 120°F/30 min. | 0/2 | 0/3 | 0/3 | 0/3 | 0/3 | 0/2 | 0 | 1/1 | 1/1 | 0/1 | 1/1 | 1/1 | 0/1 | 66 |
| 120°F/60 min. | 0/2 | 0/2 | 0/3 | 0/3 | 0/3 | 0/4 | 0 | 0/1 | 0/1 | 0/1 | 0/1 | 0/1 | 0/1 | 0 |

Since Dieffenbachia cane is 0.5–2.0 in. in diameter, it takes longer to heat the center of the large pieces than the small ones. Two experiments were made to measure the rate of heat transfer in varying sizes of cane. Centers of pieces of cane 8 in. long of varying diameters were affixed with thermocouple wires and immersed in water held at 130±1°F. The temperature was recorded at 1-minute intervals after immersion. The means of the data for the 2 experiments are shown in Fig. 2. It is significant that heat penetration through canes of 1.5 in. or less was very similar for all sizes of cane, whereas it was much slower through 2-in. cane. Thus, cane over 1.5 in. in diameter probably should not be used in hot-water treatment.

3) *Eradication of bacteria in cane with hot water.*—Since the thermal death point of the bacterium was within the range tolerated by uninfected cane, an attempt was made to eradicate the bacteria from infected cane with hot water. Stems of inoculated plants were immersed in hot water for varying times and the effectiveness determined by inoculating plants with infusions from the treated pieces. Also, portions of the stems were planted to test the adverse effects of the heat treatment.

The details were as follows: Thirty-three *D. picta* 'Rudolph Roers' plants (18–24 in. tall, 1.25–1.50 in. in diameter, 6–9 nodes) were selected, and 24 of the most uniform plants were inoculated with washings from eight 20-hour bacterial cultures growing on YDC. Each clearly defined internode was carefully inoculated by puncturing all the way through the stem. The stem punctured was swabbed again with the bacteria. Uninoculated plants were treated likewise except that sterile water was used in place of bacteria. The plants were held in a greenhouse at 70–90°F. In 3 days ooze was produced at each puncture and the remnants of leaf sheaths at the lower nodes were rotting. The stems were firm, and soft rot had not developed. The stems were cut into lengths of 4–10 in., averaging 6 in. The average diameter of the pieces was 1.0–1.15 in. The leaf sheaths were trimmed off and the stem pieces were bagged in cheesecloth and immersed in water at 120±1°F for 15, 30, or 60 minutes. A control series was treated at 80°F for 60 minutes. Uninoculated stems were treated first.

Thermocouple wires were affixed to the center of each of 3 inoculated and 3 uninoculated canes 0.5, 1.5, and 3.0 in. from the end. The temperature reached at each position at 1-minute intervals was recorded.

The canes were retrieved and cooled with running water, taking care not to contaminate the cane by handling or contacting other treatments. About 0.5 in. of each end of the cane pieces was aseptically trimmed off and the remainder cut into 4 or 5 pieces, each containing 1 or 2 nodes. One piece from the center and 1 from an end were used for subsequent subinoculation, and the remaining 2 or 3 pieces were used for propagation.

Inoculum was prepared by aseptically cutting out the infected areas of the 2 inoculum pieces, mincing in 20 ml sterile water, and steeping for 10–20 minutes. The inoculum prepared from a single treated stem was used to stem-puncture-inoculate 1 *D. picta* 'Rudolph Roers.' Six replications were used for each treatment, making a total of 30 plants. Plants were returned to the greenhouse at 70–90°F. Symptoms appeared in 5 days, notes were taken weekly for 5 weeks, and final notes were taken at 3.5 months.

To test the effect of hot-water treatment on propagation of cane, the remaining 2 or 3 pieces from each stem were placed under steamed sphagnum moss in 2-in. clay pots. Each pot contained pieces from only 1 stem, and the pots were kept spaced to reduce the chances of cross-contamination. The sphagnum was kept moist by an automatic intermittent mist appliance in a greenhouse propagation chamber at 70–80°F. Effects on rooting were noted at 9 weeks.

Temperature curves plotted from the thermocouple readings were similar to the curves for 1.5 in. diameter, shown in Fig. 2. The center of the same piece, however, did not reach 120°F until an average of 21 minutes after immersion. Consequently, it was predicted that the 30-minute treatment would be partially successful, since portions of the cane would harbor bacteria that would be subjected to less than a lethal heat dosage. The results (Table 1) confirmed the pre-

diction. The bacteria were unaffected by the 15-minute, partially reduced by the 30-minute, and eliminated by the 60-minute treatment.

The effects on rooting, as shown in Table 1, appear somewhat ambiguous at first because rooting was poorer from the control series than from several of the hot-water treatments. Mr. P. A. Chandler, of this Department, and others in commercial practice, have found, however, that hot-water treatment often stimulates rooting, even though obvious pathogens are absent. This appears to be the case here. Rooting was stimulated by the 15-minute, somewhat curtailed by the 30-minute, and almost eliminated by the 60-minute treatment. The combination of lack of control of bacteria below the 60-minute treatment and adverse effects of heat treatment above 30 minutes explains why all treatments were fatal in the inoculated series of Table 1.

*General control recommendations.—Step I.*—All obviously infested plants must be discarded and the growing area disinfested with steam or other means. Sanitary practices must be followed to reduce the inoculum potential (1).

*Step II.*—All cuttings for propagation must be immersed 15 minutes in Agri-mycin 100 (200 ppm) and rooted in steamed media in a disinfested place.

Steps I and II will reduce a great portion of the disease losses. Traces of the pathogen will survive, however, so it is necessary to follow the eradicative procedures of the next step.

*Step III.*—Select healthy-appearing cane 1.0–1.5 in. in diameter, treat in 120°F water for 40–60 minutes, root in steamed sphagnum moss in a disinfected greenhouse, and adhere strictly to sanitary measures. The time of treatment will vary. The maximum time of treatment should be consistent with the survival ability of the cane pieces. At all times the disinfested stock must be kept entirely separate from other stocks. When enough plants from these disinfested canes are produced, the old stock must be discarded. In this way, routine operations may continue, and in 1 or 2 years the grower may eradicate the bacterium from his establishment.

**Discussion.**—The nomenclature of the causal bacterium remains obscure, because of the conflicting views of various authorities (4, 7). The Dieffenbachia pathogen is very similar to *E. chrysanthemi* as described by Burkholder et al (5). Culturally, however, it differs slightly, and it is clearly different in pathogenicity. It seems wise to leave it as a form of *E. chrysanthemi* until the whole group of these Erwinias can be reworked and some agreement reached by the various workers. In this regard, the author and Professor M. P. Starr have been attempting to clarify the pathological position of these Erwinias, but it has been very difficult. Some of the isolates have become avirulent; others react in ways not noted in the original description. This phase of the work is being continued.

There is close correlation between heat penetration through cane, thermal death point of the bacterium, and eradication of the bacteria in infested cane. The fact that cane diameter so markedly influences heat penetration can explain some failures of hot-water treatment reported with other plants. In this study it was predicted and found true that treating cane 1.0–1.15 in. in diameter with 120°F for 30 minutes would be partially successful in eradicating the bacteria. Thermocouple measurements confirmed the fact that the ambient temperature of 120°F was not reached until about 22 minutes after immersion. Hence, the 30-minute treatment resulted in exposure of some of the bacteria in the cane to less than their thermal death point. Their survival was assured, and the pathogenicity tests confirmed it.

The palliative control with Agri-mycin 100, applied as a cutting dip, was remarkable. The initial recommendation was made as an educated guess before the exact cause of the disease was known. Two weeks after the treatment was begun the grower informed the author with obvious relief that an epidemic in which he was losing about $400 weekly, had stopped "entirely." As detailed above, Agri-mycin 100 was not successful as an eradicative treatment, and it was necessary to develop other controls for elimination of the pathogen.—Department of Plant Pathology, University of California, Los Angeles.

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# NONINFECTIOUS CRINKLE LEAF ON SANTA ROSA PLUM

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Cooperative investigations between Crops Research Division, ARS, U. S. D. A., and University of California Citrus Experiment Station, Riverside.

SUMMARY

Transmission tests and field observations show that Santa Rosa plum crinkle leaf is bud-perpetuated but not graft-transmissible. The disorder somewhat resembles sweet cherry crinkle leaf and prune crinkle leaf, but no relationship is known. It is concluded that Santa Rosa crinkle leaf is caused by a genetic instability.

**Introduction.**—Prune crinkle leaf and sweet cherry crinkle leaf have been recognized for many years in orchards of the western United States. Despite their viruslike appearance, neither disorder has been transmitted to healthy trees. Prune crinkle leaf has been observed in Italian Prune, Emilie, and Hungarian prune and certain other types of *Prunus domestica* L. (1). Sweet cherry crinkle leaf is most common in Bing and Black Tartarian, but has been reported in several other varieties of *P. avium* L. (3). Both disorders are of considerable economic importance since affected prune and sweet cherry trees are generally unfruitful. Annual losses are great in commercial orchards as well as in nursery stock.

A severe disorder, analogous in many ways to prune crinkle leaf and sweet cherry crinkle leaf, has been observed more recently in Santa Rosa plum trees (*P. salicina* Lindl.) in the Yucaipa valley, southern California. The few observed trees, in 3 orchards, are affected entirely or in a single branch.

**Fig. 1.**—Leaves from a Santa Rosa plum tree affected with the plum crinkle leaf disorder.

**Symptoms.**—Affected portions are conspicuously dwarfed. The leaves are ⅓–½ normal size and distorted in a variety of patterns: strap-shaped, pronounced lobes along the margins, or severely indented margins (Fig. 1). Some leaves display a pale yellow pattern along the margins or a mottle spreading over the whole blade. Leaves on affected branches are thickened and tend to become brittle early in the growing season. Leaf midveins are enlarged, and the entire leaf venation is irregularly formed. Leaves produced early in the growing season are always deformed; later leaves may be nearly normal. A few blossoms may appear on affected branches, but they are imperfectly formed. Affected branches and trees are almost entirely unfruitful. Many leaf and fruit buds abort, so that affected trees appear sparse and stringy (Fig. 2).

**Transmission tests.**—Although plum crinkle leaf is readily bud-perpetuated, all attempts to transmit it have failed. In 1954, a grower in the Yucaipa area cut back two affected 8-year-old Santa Rosa trees and grafted healthy Santa Rosa scions to the stumps. The new frameworks are healthy in every respect and produce a commercial crop. Suckers growing from the stumps, however, have been severely affected with plum crinkle leaf each year. In 1954, scionwood from affected orchard trees was grafted to 4 healthy Santa Rosa trees growing in an experimental plot at Riverside. Growth from these grafts has perpetuated the disorder, but no transmission has been noted. Subsequent attempts to transmit crinkle leaf through bud inoculations to healthy Santa Rosa nursery trees have all failed, but growth from the inserted bud has always shown symptoms of the disorder. When buds were taken from healthy-appearing portions of affected orchard trees and placed in young Santa Rosa trees, no symptoms of crinkle leaf appeared in the test trees or in the growth from the inoculum buds. Since affected trees or branches are almost completely unfruitful, it has been impossible to determine whether this condition is seedborne.

**Fig. 2.**—Branch from a Santa Rosa plum tree affected with plum crinkle leaf. Healthy branch at the right.

**Discussion and conclusion.**—Of greater importance than the striking differences between the three described crinkle leaf disorders is the over-all similarity of symptoms, especially the general sterility of affected branches, the distortion of venation and leaf shape, and the failures in transmission.

Varying degrees of mosaic mottling, somewhat similar in pattern, are common in leaves of all three. In general, affected Santa Rosa plum trees are severely dwarfed whereas prune or sweet cherry trees with crinkle leaf show little or no dwarfing. Crinkle-leaf-affected Santa Rosa plum trees are more nearly unfruitful. Affected prune trees vary in unfruitfulness, but some may set a small crop that tends to fall before maturity. Crinkle-leaf-affected sweet cherries usually set a few fruits, but these are small and pointed and hang with the apex out of line with the stem. The rare fruits that set on affected Santa Rosa plums are also small and pointed and hang off-center. Leaf distortion is more pronounced and striking on affected Santa Rosa plum trees than on prune and cherry trees, and is apparent from the beginning of growth; the initial leaves on affected prunes and cherries are sometimes normal in appearance.

Santa Rosa plum in general seems more completely and strikingly affected than prunes or sweet cherries. Crinkle leaf is widespread in Italian Prune; so is crinkle leaf in sweet cherry, particularly in western United States. Crinkle leaf in Santa Rosa plum appears to be restricted to a few scattered trees in California plus a few cases in the State of Washington (reported by E. L. Reeves in correspondence with the authors).

The cause of crinkle leaf remains obscure. Brooks and Hewitt (2) showed that sweet cherry crinkle is both seed- and pollen-transmitted. Since it cannot be transmitted through tissue grafts, virus must be ruled out as an etiological agent. Environment is ruled out by the occurrence on a variety of soils, in a variety of climates, and in locations adjoining normal trees. The most plausible cause of the disorder seems to be a genetic instability in certain of the tissues. When these become apical the resulting growth is affected.

Where several varieties of a given host, such as sweet cherry, are affected, they may have a common factor for the genetic instability in their inheritance, but where such distantly related species as sweet cherries, prunes, and Santa Rosa plum are affected, separate unrelated factors must be postulated. Although prune crinkle is known on several varieties of *P. domestica*, this is the first report of the disorder on *P. salicina*.—U. S. Department of Agriculture, Citrus Experiment Station, Riverside, California.

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# INFLUENCE OF VARIOUS FACTORS ON THE DETERIORATION OF STORED CORN BY FUNGI

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SUMMARY

High-quality yellow dent corn was stored at moisture contents of 12–18%, at temperatures of 5–30°C, not inoculated and inoculated with different species of storage fungi alone and in mixture, and tested at intervals up to 2 years for percentage germination, percentage of discolored germs, and number and kinds of fungi. Heavy germ damage (brown, decayed germs) occurred in grain inoculated with storage fungi and kept at 14% moisture content and 30°C for 2 years, and moderate to heavy germ damage occurred in grain inoculated with storage fungi and kept for 2 years at 17–18% moisture content and 10–15°C. Noninoculated controls remained free of fungi; after 2 years at 14% moisture content and 30°C, germination was 88%, and after 2 years at 17–18% moisture content and 15°C, germination was 96%. No brown germs developed in any of the fungus-free controls. Deterioration as judged by decrease in germination, increase in germ damage and increase in storage fungi was much greater in lots of grade no. 2 corn from commercial bins than in the seed grade corn stored under the same conditions. Injuries to the pericarp over the germ facilitated entry of storage fungi into the kernel. Moisture content and temperature of the stored grain, the degree to which it has previously been invaded by given species of storage fungi, and pericarp injuries all act together to influence the rate of deterioration of a given lot of corn stored under conditions that permit storage fungi to grow.

**Introduction.**—A previous paper (7) summarized the effects of fungi on the deterioration of corn stored for a few months at moisture contents of 12–18% and temperatures of 5–25°C. The present paper reports additional studies on the factors involved in damage caused to corn by storage fungi.

**Materials and methods.**—*Corn.*—The seed quality corn used in most of the tests was grown at St. Paul, Minnesota. Mature ears were harvested by hand, dried immediately, shelled, cleaned, and kept under refrigeration until used. When surface disinfected and cultured on various agar media Alternaria and Fusarium grew from about 1% of the kernels, but no storage fungi grew from them. Samples of grade no. 2 corn from a local terminal elevator were used in one of the tests; their storage histories were not known.

*Moisture content.*—The moisture content of the grain, determined by the one-stage or two-stage air-oven method specified in Cereal Laboratory Methods (1), is given on a wet-weight basis.

*Germination percentage.*—One hundred kernels were placed on moist paper towels, and these were wrapped in waxed paper and kept 9 days at room temperature; germinated seeds were counted at intervals of 3 days.

*Germ color.*—One hundred kernels were cut lengthwise through the middle of the embryo and examined with a stereoscopic microscope and with the naked eye. Germ color was rated as normal, ochre, or brown. Those rated brown corresponded to those designated in the grain trade as "germ damaged."

*Fungus infection.*—Fifty to 100 kernels were shaken 1-1.5 minutes in 1% sodium hypochlorite plus 10 ppm of Tween-20, rinsed in sterile water, cultured on malt agar containing 7.5% sodium chloride, and incubated 5–10 days, and the fungi which grew from them were identified. For mold counts, 10–20 g of grain (50–100 kernels) were comminuted 1.5 minutes in a Waring blender containing 500 ml of sterile 0.2% agar in water, further dilutions were made in the same medium, 1-ml portions of one or more suspensions put in each of 2 Petri dishes, malt-salt agar cooled to 50–52°C added, and the dishes swirled to distribute the material uniformly, and incubated until the colonies could be counted and identified. These 2 procedures have been used extensively to determine the number and kinds of storage fungi present on and in grains (2, 4, 6, 7).

*Preparation of samples for storage.*—Portions of 1000 g of corn were shaken 1–1.5 minutes in 1% sodium hypochlorite solution, rinsed twice in sterile water, placed in sterile paper bags, and dried at 30°C in a forced-air oven. Moisture content was determined, and sterile water added to attain the moisture content

**Table 1.**—Influence of moisture content (m. c.) and temperature on germination, germ discoloration, and fungus invasion of yellow dent corn not inoculated and inoculated with a mixture of Aspergillus and stored 2 years.*

| | Temperature | Final m.c. | Germination | Discoloration | | Surface-disinfected kernels yielding | | |
|---|---|---|---|---|---|---|---|---|
| | | | | Brown | Ochre | *A. glaucus* | *A. candidus* | *A. flavus* |
| Original m.c. 16%: | | | | | | | | |
| inoculated | 5 | 15.6 | 96 | 0 | 0 | 54 | 0 | 0 |
| | 10 | 15.6 | 90 | 0 | 0 | 62 | 0 | 0 |
| | 15 | 15.9 | 48 | 0 | 3 | 100 | 0 | 0 |
| not inoculated | 5 | 15.7 | 100 | 0 | 0 | 0 | 0 | 0 |
| | 10 | 15.8 | 100 | 0 | 0 | 0 | 0 | 0 |
| | 15 | 15.6 | 100 | 0 | 0 | 0 | 0 | 0 |
| Original m.c. 18% | | | | | | | | |
| inoculated | 5 | 17.3 | 67 | 0 | 0 | 100 | 0 | 0 |
| | 10 | 17.5 | 32 | 12 | 18 | 100 | 0 | 0 |
| | 15 | 17.8 | 0 | 68 | 20 | 94 | 52 | 16 |
| Not inoculated | 5 | 17.8 | 100 | 0 | 0 | 12 | 0 | 0 |
| | 10 | 17.1 | 82 | 0 | 6 | 28 | 0 | 0 |
| | 15 | 17.0 | 96 | 0 | 0 | 6 | 0 | 0 |

* All figures are %, except for the temperature column (°C).

(m.c.) desired for the test, and the grain kept several days in sterile jars in a refrigerator and shaken occasionally to distribute moisture uniformly. Portions of 250–500 g were then placed in autoclaved pint or quart Mason jars equipped with rubber rings and screw caps, inoculated, and stored in incubators. In some tests no free water was added; the corn with or without preliminary surface disinfection was placed in open dishes above saturated salt solutions in desiccators and allowed to come to equilibrium m.c. Inoculum consisted of dry spores of the various storage fungi that had been isolated from commercially stored corn and grown on autoclaved wheat.

**Results.**—*Inoculations of seed quality corn with mixtures of storage fungi.*—Samples of seed quality corn prepared as described above were adjusted to moisture contents of 12, 14, 16, and 18%, inoculated with a mixture of spores of *Aspergillus amstelodami* (Mang.) T. & C., *A. repens* (Cda.) DeBary, *A. ruber* (Spiek and Brem.) T. & C., (these 3 are in the *A. glaucus* group); *A. candidus* Link, *A. flavus* Link, and *A. ochraceus* Wilh., stored at various temperatures and tested after 2 years. Those at 12 and 14% m.c. were stored at temperatures of 5, 10, 15, 20, and 25°C, while those at 16 and 18% m.c. were stored only at temperatures of 5, 10, and 15°C. Previous tests (7) had shown that in corn stored at m.c. of 16 and 18% and temperatures of 20 and 25°C any of the fungi used were capable of causing extensive deterioration within 3–6 months. Hence, in the present tests only those combinations of m.c. and temperatures were used that were expected to span the range between low and high deterioration in a 2-year period.

The corn stored at 12% m.c. and 5–25°C retained a germination of 96–100% after 2 years. That stored at 14% m.c. and at temperatures of 5–20°C germinated almost 100% after 2 years, although a few kernels of the samples at 15 and 20°C were invaded by *A. repens*. After storage at 14% m.c. and 25°C for 2 years, 62% of the kernels yielded *A. repens* and the germination was 56%; the germs of a number of kernels were off color but none were dark enough to be rated either ochre or brown.

The results of storage for 2 years at m.c. of 16 and 18% and 5, 10, and 15°C are given in Table 1. The m.c. of all samples decreased slightly during the storage period. The corn at 16% m.c. was invaded only by members of the *A. glaucus group;* no germ discoloration was evident in the grain stored at 5 and 10°C, but 3% of the kernels at 15°C had ochre-colored germs. The noninoculated grain remained free of fungi, retained a germination of 100%, and developed no discolored germs.

Stored at 18% m.c. for 2 years, 100% of the inoculated kernels were invaded by fungi, even at 5°C, although no germ damage developed at that temperature. *A. repens* was the main or only fungus recovered from the surface-disinfected kernels after they had been stored at 5 and 10°C, but *A. candidus* grew from 52% and *A. flavus* from 16% of the surface-disinfected kernels after storage for 2 years at 15°C. In the sample at 10°C 12% of the inoculated kernels had brown germs, and at 15°C 68% of the inoculated kernels had brown germs and the germination was zero. The noninoculated corn stored at 15°C and 18% m.c. (final m.c. 17%) germinated 96% and none of the germs was discolored. Typical seedlings from the noninoculated sample are shown in Fig. 1. This is rather convincing evidence that, in the absence of storage fungi, corn stored at 17–18% m.c. and 15°C will not deteriorate appreciably in 2 years.

*Inoculation of seed quality corn with members of the A. glaucus group.*—In the test described above, *A. repens* invaded some of the corn stored at 14% m.c. for 2 years. A similar test was made, with corn prepared as above and adjusted to a moisture content of 14%, inoculated with spores of 5 common members of the *A. glaucus* group, stored at 20 and 30°C and

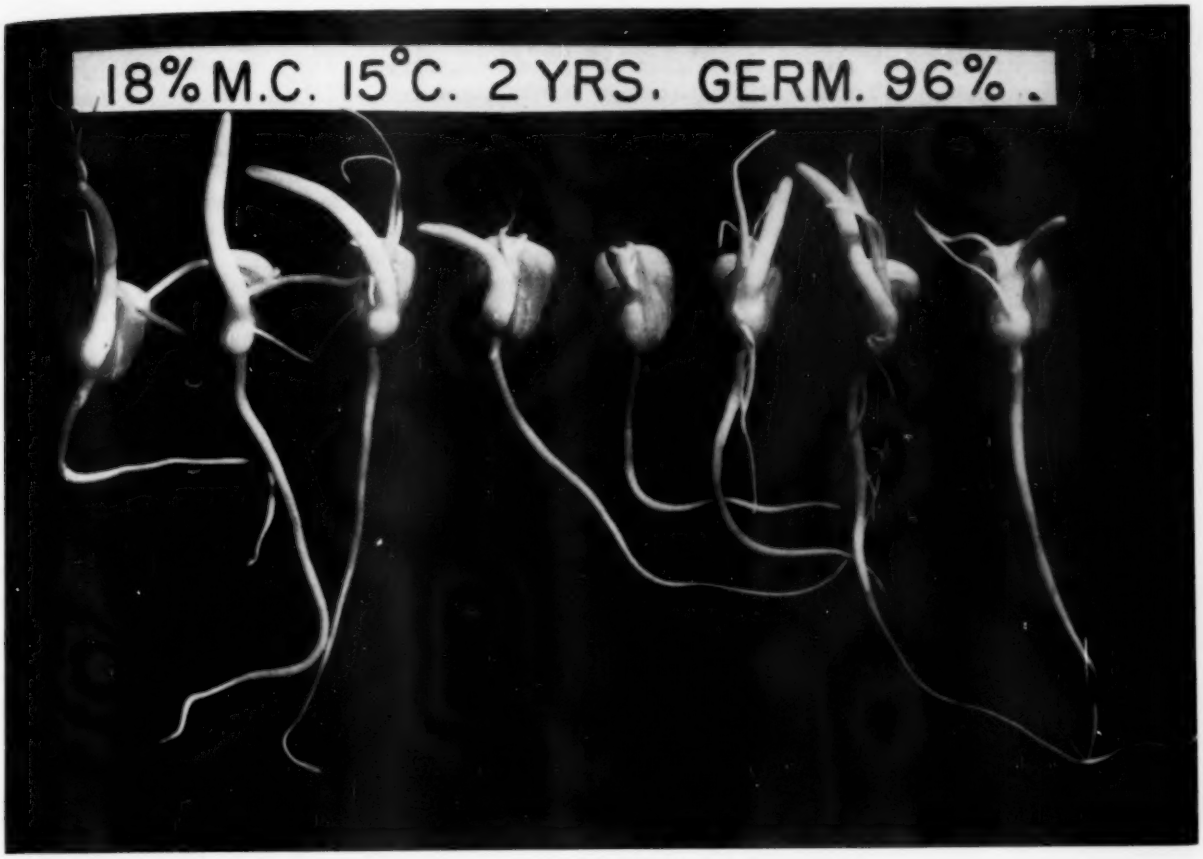


**Fig. 1.**—Germination 96% in corn stored 2 years at 15°C and 18% moisture content.

tested after 2 years. The results are given in Table 2.

A considerable number of the kernels stored at 20°C were invaded by the different fungi, but germination percentage was not significantly reduced and no brown germs were detected. Stored at 30°C for 2 years, however, the germination percentage of kernels inoculated with 4 of the 5 species of fungi was reduced to zero, while the percentage of discolored germs (brown and ochre together) ranged from 6% in the grain inoculated with *A. amstelodami* to 90% in that inoculated with *A. ruber*, and 98% in that inoculated with *A. chevalieri*. A typical example of mycelium growing from a discolored germ is shown in Figure 2.

The noninoculated grain stored at 14% m.c. and 30°C for 2 years retained a germination of 88%, and had no discolored germs. This again is convincing evidence that high-quality corn in the absence of storage fungi will not undergo much deterioration

**Table 2.**—Effect of different members of the Aspergillus glaucus group on germination, germ discoloration, and fungus invasion of seed-grade yellow dent corn stored 2 years at 14% moisture content[a] and 20 and 30° C.

| *Inoculation* | Temperature (°C) | Germination (%) | % germs discolored | | % surface-disinfected kernels yielding inoculated species |
|---|---|---|---|---|---|
| | | | Brown | Ochre | |
| *A. amstelodami* | 20 | 97 | 0 | 4 | 72 |
| | 30 | 22 | 0 | 6 | 66 |
| *A. chevalieri* | 20 | 95 | 0 | 6 | 44 |
| | 30 | 0 | 64 | 34 | 96 |
| *A. repens* | 20 | 98 | 0 | 2 | 32 |
| | 30 | 0 | 6 | 18 | 62 |
| *A. ruber* | 20 | 97 | 0 | 4 | 22 |
| | 30 | 0 | 29 | 61 | 94 |
| *A. umbrosus* | 20 | 98 | 0 | 2 | 4 |
| | 30 | 0 | 14 | 57 | 73 |
| Not inoculated | 20 | 99 | 0 | 0 | 0 |
| | 30 | 88 | 0 | 0 | 0 |

[a] Final moisture contents ranged from 13.6 to 14.1%.

when stored 2 years at 14% m.c. and 30°C. If storage fungi are present, as they almost inevitably are on and in commercially stored grain, a combination of a storage temperature of 30°C and 14% m.c. over a period of 2 years may result in extensive damage, with consequent reduction in market grade and price.

*Inoculation of high-quality corn with additional species of Aspergillus.*—Samples of seed quality corn prepared as described above were adjusted to different moisture contents and inoculated separately with *A. restrictus*, *A. ruber*, *A. candidus*, and *A. flavus*, stored at temperatures of 15–30°C, and tested after different time intervals. The results are summarized in Table 3. Only those moisture contents, temperatures, and storage times are given in the table that serve to illustrate the major differences found. That is, tests with *A. flavus* included storage periods of 1.5, 3, 4.5, and 6 months at both 15 and 25°C, but only the results after 4.5 months are tabulated because that is where the difference between 15 and 25°C was greatest. Similarly, samples inoculated with *A. candidus* were stored at higher and lower moisture contents and also at lower temperatures than shown in the table, and tested at several intervals up to 8 months; but the results of storage at only 1 moisture content (15.5–15.7%) and 1 storage period (8 months) are given because that is where the differences in germination percentage and in percentage of discolored germs were greatest.

The germination percentage of corn stored at 15 and 20°C was reduced considerably in 4.5–16 months, and some germs became ochre-colored but none became dark brown. The corn inoculated with *A. flavus* and stored at 16% m.c. and 25°C was reduced some in germination percentage, and 2% of the kernels developed ochre germs, but this probably was due to contamination by *A. repens*, since *A. flavus* was recovered from only 3% of the surface-disinfected kernels. The data in Table 1, as well as previous evidence (7), indicate fairly conclusively that *A. flavus* cannot invade corn with a moisture content less than about 18%. In the corn stored at 15–17% m.c. and 30°C, the germination percentage after 6–16 months was zero, even in the noninoculated controls. No discolored germs were detected in the controls, but ranged from 40 to 92% (brown and ochre germs together) in the samples inoculated with the different fungi. In corn stored at 15.3% m.c. and 20°C, *A.*

**Fig. 2.**—Mycelium growing from the discolored germ of a sectioned corn kernel cultured on malt-salt agar. The corn had been inoculated with *Aspergillus ruber* and stored 2 years at 14% moisture content and 30°C. No sporophores or mycelium were visible on the outside of the kernels after storage for 2 years, but the germs were heavily invaded by *A. ruber*.

**Table 3.**—Influence of moisture content, temperature, storage period, and species of fungus on germination and germ discoloration of stored yellow dent corn inoculated with *Aspergillus*.

| Inoculation | Moisture content (%) | Temperature (°C) | Months stored | Germination (%) | % germs discolored | | % surface-disinfected kernels yielding fungi inoculated |
|---|---|---|---|---|---|---|---|
| | | | | | Brown | Ochre | |
| *A. candidus* | 15.5 | 20 | 8 | 100 | 0 | 0 | 100 |
| | 15.7 | 30 | 8 | 0 | 0 | 40 | 100 |
| *A. flavus* | 16 | 15 | 6 | 100 | 0 | 0 | 0 |
| | 16 | 25 | 6 | 82 | 0 | 2 | 38* |
| | 18 | 15 | 4.5 | 62 | 0 | 9 | 100* |
| | 18 | 25 | 4.5 | 0 | 94 | 2 | 100 |
| *A. restrictus* | 15.3 | 20 | 16 | 48 | 0 | 6 | 100 |
| | 15.4 | 30 | 16 | 0 | 86 | 6 | 100 |
| *A. ruber* | 15.1 | 20 | 9 | 85 | 0 | 3 | 100 |
| | 15.3 | 30 | 9 | 0 | 50 | 26 | 100 |
| | 16.7 | 20 | 6 | 56 | 0 | 20 | 100 |
| | 16.7 | 30 | 6 | 0 | 55 | 37 | 100 |
| Not inoculated | 15.0 | 20 | 9 | 97 | 0 | 0 | 0 |
| | 15.0 | 30 | 9 | 0 | 0 | 0 | 0 |
| | 16.8 | 20 | 6 | 95 | 0 | 0 | 0 |
| | 16.3 | 30 | 6 | 0 | 0 | 0 | 0 |

* Surface-disinfected kernels yielded *A. repens*, which evidently entered as a contaminant.

*restrictus* in 16 months reduced the germination to 48%, and 6% of the kernels developed ochre germs, while in the corn at the same moisture content but at 30°C, 86% of the germs became brown and 6% ochre. In corn stored at 18% m.c. and 15°C, *A. flavus* produced no brown germs in 4.5 months, while at 25°C 94% of the germs were brown. No discolored germs were detected in the controls, even in those stored at 30°C in which the germination was reduced to zero. Evidently a temperature of 30°C is lethal to corn stored some months at a moisture content of 15% and above, although (see Table 2) corn stored at 14% m.c. and free of fungi for 2 years at 30°C decreased little in germination. The development of the dark color typical of germ damage appears to be a product of infection by storage molds and a temperature above 20°C. All of these factors must be considered together if one is to evaluate the deterioration risk to which a given bulk or parcel of corn is likely to be exposed under a given set of storage conditions. As will be brought out in the next section, the condition of corn as it goes into a given bin may likewise greatly affect its storage behavior.

*Deterioration of commercial lots of grade no. 2 corn compared with that of seed quality corn.*—Some evidence already has been presented (7) to show that commercial samples of grade no. 2 corn deteriorate more rapidly than seed quality grain when stored under the same conditions. The same has been shown to be true of wheat (8). In the tests previously reported (7) the samples of grade no. 2 corn from commercial bins and the seed quality corn with which they were to be compared were stored for 30 days at 18% m.c. and 25°C. In the present tests the same lots were stored at 16% m.c. and tested after 2 and 4 months. The results are given in Table 4.

After 2 months the germination percentage of the 3 commercial samples ranged from 1 to 12% and the percentage of discolored germs (brown and ochre together) ranged from 16 to 46%. The seed quality corn germinated 98%, 2% of the germs were ochre colored, and none of the germs was brown. After 4 months the germination of the commercial samples was zero, and discolored germs ranged from 42 to

**Table 4.**—Germination, germ discoloration, and fungus invasion of 3 commercial samples of grade no. 2 yellow dent corn and 1 sample of seed-grade yellow dent corn stored 2 and 4 months at 16% moisture content and 25°C.

| Sample | Months stored | Germination (%) | % germs discolored | | % surface-disinfected kernels yielding *Aspergillus glaucus* | Mold count (thousands) |
|---|---|---|---|---|---|---|
| | | | Brown | Ochre | | |
| Commcl. A | 0 | 50 | 1 | 16 | 40 | 7.5 |
| | 2 | 12 | 2 | 14 | 80 | 60 |
| | 4 | 0 | 16 | 26 | 100 | 442 |
| Commcl. B | 0 | 62 | 2 | 11 | 55 | 11.5 |
| | 2 | 1 | 6 | 28 | 100 | 350 |
| | 4 | 0 | 18 | 46 | 100 | 960 |
| Commcl. C | 0 | 48 | 5 | 22 | 46 | 81 |
| | 2 | 6 | 10 | 36 | 100 | 700 |
| | 4 | 0 | 28 | 58 | 100 | 1200 |
| Seed-grade | 0 | 98 | 0 | 0 | 4 | 4 |
| | 2 | 98 | 0 | 2 | 48 | 48 |
| | 4 | 69 | 4 | 14 | 100 | 92 |

**Table 5.**—Influence of scarification on germination, germ discoloration, and fungus invasion of yellow dent corn inoculated with a mixture of the *Aspergillus glaucus* group and stored 2 months at 85% relative humidity and 25°C.

| Sample and treatment | Final moisture content (%) | Germination (%) | % germs discolored | | % surface-disinfected kernels yielding | | Mold Count (thousands) | |
|---|---|---|---|---|---|---|---|---|
| | | | Brown | Ochre | *A. candidus* | *A. glaucus* | *A. candidus* | *A. glaucus* |
| Scarified | | | | | | | | |
| A | 17.1 | 6 | 10 | 22 | 69 | 100 | 720 | 390 |
| B | 17.2 | 7 | 32 | 8 | 46 | 100 | 240 | 740 |
| Not scarified | | | | | | | | |
| A | 17.0 | 34 | 1 | 14 | 24 | 100 | 200 | 260 |
| B | 17.0 | 69 | 4 | 8 | 24 | 100 | 80 | 260 |

86%; the seed quality corn germinated 69%, 4% of the germs were brown and 14% ochre. Under conditions that permit storage fungi to grow, corn as ordinarily encountered in commercial storage often can be expected to deteriorate much more rapidly than the high quality seed corn used in most of our tests.

*Influence of pericarp injuries on fungus invasion and deterioration of stored corn.*—Koehler (5) examined many samples of seed corn over a period of 10 years and found that an average of 78% of the kernels had pericarp injuries detectable by the method he used (staining with a 0.1% aqueous solution of fast green). Using the same method we examined a number of samples of corn from commercial storage, and found pericarp injuries to be very common. Two lots were chosen for further tests; one of these (A) had detectable injuries to the pericarp over the embryo on 33% of the kernels, the other (B) had such injuries on 13% of the kernels. A portion of each lot was scarified by shaking the grain briskly in a tin can through the bottom of which a nail had been driven many times from the outside inward, leaving many sharp protrusions on the inner side. This increased the visible injuries in that portion of the pericarp covering the embryo to 95% of the kernels in lot A, and to 82% of the kernels in lot B. Scarified and nonscarified portions were inoculated with a mixture of spores of *A. amstelodami*, *A. repens*, *A. restrictus*, *A. ruber*, and *A. candidus*, stored in open dishes in a desiccator containing a saturated solution of KCl to maintain a relative humidity of 85%, at 25°C, and tested after 2 months. The results are given in Table 5.

The differences between the 2 nonscarified lots were not great, except in germination percentage, which was lower in lot A, which had injuries to the pericarp over the embryo of 33% of the kernels than in lot B, which had such injuries on 13% of the kernels. The number of colonies of *A. candidus* also was considerably higher in lot A than in lot B.

The differences between the scarified and nonscarified portions of each lot were large: the germination percentage of the scarified grain was much lower than that of the nonscarified, percentage of discolored germs much higher, percentage of surface-disinfected kernels yielding *A. candidus* was from 2–3 times as great, and the mold count 2–3 times as great. We conclude from this that cracks, breaks or scratches in the pericarp over the germ furnish a ready entry port for the storage fungi. Evidence not presented here indicates that if the pericarp of corn kernels is unbroken, storage fungi grow into the germ through the point of attachment, and almost only there, much as they enter cottonseed (3). The pericarp itself appears to be very resistant to penetration by storage fungi; this is obvious, of course, in the commonly encountered condition known as "blue eye," in which fungi that have invaded the embryo produce masses of spores beneath the pericarp and yet do not sporulate on the outside unless breaks in the pericarp allow the spores to grow out.

**Discussion.**—The rate at which corn deteriorates in storage is influenced greatly by a number of factors, of which the most important appear to be moisture content, temperature, the kinds of fungi involved, the length of time and rate at which the fungi grow, the condition of the grain, and the number and perhaps location and severity of injuries in the pericarp of the corn kernels. These factors operate together, at least in corn as it ordinarily is encountered in commercial storage, and so must be considered together. This probably explains why some lots of corn can be stored at a moisture content of 13–14% for several years without an appreciable amount of germ damage developing, while in others, stored at essentially the same moisture content and temperature, extensive germ damage occurs; or why some corn bulks stored at 15% m.c. and 50°F retain for a long time the same condition as when they were binned, while others after a short time undergo molding and heating.

The lowest moisture content at which storage fungi invaded the corn and caused extensive germ damage in the present tests was 14%, and then only when the corn was stored at 30°C for 2 years. However, the corn used in these tests was of seed quality; had samples of grade no. 2 corn from commercial bins been included, it is possible that fungus invasion and damage would have occurred at a temperature lower than 30°C or at a moisture content lower than 14%. Also, when these tests were begun *A. restrictus* had not been encountered frequently on stored corn, and so it was not included in the tests that extended over a 2-year period. *A. restrictus* has since been found

to be prevalent in some bulks of corn that have been stored for a year or more, and we have found it heavily invading, as almost a pure culture, and causing heavy germ damage in some tens of thousands of bushels of corn stored at a moisture content from just below to just above 14%. More extensive tests are needed with different isolates of *A. restrictus*, to determine the lower limits of moisture content and temperature at which this species will cause commercially important damage.

Temperature is just as important as moisture content in determining the amount of damage likely to develop in stored corn. At 16% m.c., no damage developed in 2 years in seed quality corn inoculated with a mixture of storage fungi and kept at 15°C or below. At 17–18% m.c. no damage developed in the corn stored at 5°C for 2 years, although the grain was invaded by members of the *A. glaucus* group. At 10 and 15°C extensive damage developed in 2 years. The corn free of storage fungi retained a germination of 96% after storage for 2 years at 17–18% m.c. and 15°C; stored at 14% m.c. and 30°C for 2 years it retained germination of 88%. No brown germs developed in any of the fungus-free controls at any of the moisture contents and temperatures tested.

The samples of grade no. 2 corn from commercial bins deteriorated much more rapidly at 16% m.c. than did the corn of seed quality. This probably reflected the degree to which the samples of grade no. 2 corn had been invaded by fungi during their storage life, and possibly also the extent of pericarp injuries. As shown previously (7) in tests of about 150 different commercial lots of corn, many had undergone some deterioration during storage. Given conditions for additional deterioration such lots will develop more extensive germ damage, and do so more rapidly, than will really sound corn.

The major cause of germ damage in corn in ordinary commercial storage is the invasion of germs by storage fungi. The dark brown germs typical of so many cases of germ damage encountered in commercial lots of corn appear to be produced mainly by a combination of invasion by any one of a number of storage fungi plus a comparatively high temperature. A knowledge on the part of grain men of the degree of invasion of given lots of corn by these storage fungi, as well as more accurate knowledge of and better control of both moisture content and temperature in bulks of stored corn would aid greatly in reducing or avoiding losses caused by storage fungi.—Department of Plant Pathology and Botany, University of Minnesota, Institute of Agriculture, St. Paul 1.

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# ADDITIONAL EVIDENCE THAT SWEETPOTATO MOSAIC VIRUS IS A STRAIN OF TOBACCO MOSAIC VIRUS

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SUMMARY

Rodlike particles were characteristic of electron micrographs made from semipurified leaf extract preparations of tobacco plants, *Nicotiana tabacum*, artificially infected with the sweetpotato mosaic virus (SMV). The particles appeared to be essentially identical morphologically with tobacco mosaic virus (TMV) particles. When TMV antiserum was overlaid with SMV antigen, a positive ring precipitin test resulted. This was also true when the SMV antiserum was overlaid with TMV antigen. It thus appears that SMV and TMV are closely related serologically, the first probably a strain of the second.

Elmer (1, 2) presented considerable evidence (host range, comparative symptoms, cross-protection data, and some other characteristics) that sweetpotato mosaic is caused by a strain of tobacco mosaic virus (TMV). These serological and electron microscopic studies were conducted to obtain additional evidence.

**Materials and methods.**—In the electron microscopic study, sweetpotato mosaic virus (SMV) from infected tobacco plants, *Nicotiana tabacum* L. var. Havana 38, was furnished by Dr. O. H. Elmer or was obtained from plants inoculated by the authors with techniques described by Elmer (1, 2). Leaves were removed from

**Fig. 1.**—Electron micrograph of TMV-like particles, which were characteristic of all sweetpotato mosaic virus preparations (chromium shadowed; ×29,000).

the diseased plants, frozen, and ground thoroughly with enough distilled water for a 1:10 extract by weight. These extracts were usually frozen overnight; after thawing at room temperature, they were centrifuged 30 minutes at low speed (3500 G). The coarse pellet was discarded and the supernatant was centrifuged 3 hours at 40,000 G in a Servall vacuum-type angle centrifuge, Type SS-2. Several series of alternate low- and high-speed centrifugations were used occasionally, but the single series was usually sufficient to obtain satisfactory semipurified preparations. The supernatant was then discarded, and the virus-containing pellet remaining in each tube was resuspended in either 1 ml of distilled water or 0.1*M* neutral phosphate buffer. These resuspended pellets were occasionally lumped and recentrifuged 30 minutes at low speed (3500 G), but the resuspended pellets usually were already clean enough to be used in electron microscopic preparations.

This resuspended pellet virus preparation was then sprayed in a fine aerosol mist by a DeVilbiss glass nebulizer no. 40 onto electron microscope grids covered with a film of 2% parlodion in amyl acetate. A micropipette was occasionally used instead of the nebulizer to place a small drop of the virus preparation on the grid, but the aerosol technique usually produced better grid preparations with better distribution of particles. After drying, the grids were shadowed with chromium at an angle of about 15°, after which they were examined in an RCA-type EMU electron microscope. In each of 8 trials, control preparations from healthy tobacco leaf were also prepared for examination, using identical techniques.

In the serology tests antiserum was prepared, using standard serological procedures, by injecting antigen (the semipurified SMV preparation described previously) intravenously into a rabbit every 5th day for 1 month. After 10 days the blood was collected, coagulated 4 hours at 37°C, and stored overnight in a refrigerator. The serum was then removed with a pipette and centrifuged at low speed to remove any blood cells and their fragments. This SMV antiserum was then stored in the refrigerator for future use.

The familiar precipitin ring test was used in 3 trials, with micro test tubes and micropipettes. The antisera were placed in each tube first and then carefully overlaid with a fixed amount of antigen of a given dilution. Antisera were undiluted in each test, whereas the antigen (either the semipurified SMV preparations previously discussed or a TMV antigen obtained from Dr. Ellen L. Moorhead, University of Nebraska) was diluted serially in 2-fold dilutions.

**Results.**—Fig. 1 shows the type of rodlike particles characteristic of the virus preparations as seen under the electron microscope. These were never seen in the control preparations. The particles varied from about 400 to 5000 Å long (mean close to 3000 Å) and were 408±29 Å in diameter. This diameter is considerably greater than that usually listed for TMV (3) but can

**Table 1.**—Precipitin ring tests showing a close serological relationship between sweetpotato mosaic virus and tobacco mosaic virus.[a]

| Antigen dilutions | Antigen-antiserum combinations | | | |
|---|---|---|---|---|
| | Antigen-SMV Antiserum-TMV | Antigen-TMV Antiserum-SMV | Antigen-healthy tissue Antiserum-SMV | Antigen-Saline Antiserum-SMV |
| 1/10 | ++++ | ++++ | — | — |
| 1/20 | ++++ | ++++ | — | — |
| 1/40 | +++ | +++ | — | — |
| 1/80 | +++ | ++ | — | — |
| 1/160 | ++ | — | — | — |
| 1/320 | ++ | — | — | — |
| 1/640 | — | — | — | — |

[a] Antisera were undiluted in all tests. Antigens were diluted to compare flocculation characteristics of different antisera. All dilutions were in physiological saline. "+" indicates flocculation or a positive ring; "—" indicates no flocculation or ring.

be accounted for (Prof. Paul Kaesberg, University of Wisconsin, personal communication) by the tendency of chromium to deposit in much thicker layers than other common heavy metals used in shadowing virus particles.

Aliquots of each virus preparation for the electron microscope were used for infectivity tests on Havana 38 tobacco. In the 8 trials each virus perparation was infectious and produced symptoms on tobacco indistinguishable from the original SMV symptoms described by Elmer (1, 2). None of the healthy control preparations were infectious.

In the serological tests, antiserum prepared by the authors was compared in 3 trials with the TMV antiserum obtained from Moorhead.

Results of a typical trial are presented in Table 1. The TMV antiserum was overlaid with SMV antigen at the various dilutions. A positive ring flocculation occurred in all dilutions up to 1:320 but not beyond. When SMV antiserum was overlaid by the various dilutions of TMV antigen, a positive ring flocculation occurred in all dilutions up to 1:80 but not beyond. The saline and healthy tobacco plant antigen controls produced no positive tests at any dilution.—Department of Botany and Plant Pathology, Kansas State University, Manhattan.

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# PROPERTIES OF WHITE CLOVER MOSAIC VIRUS FROM INDIANA

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SUMMARY

The Indiana isolate of white clover mosaic virus is mechanically transmissible to 26 species in 5 families. The virus retains its infectivity at dilutions up to about $1\times10^{-6}$, at temperatures (10-minute exposure) up to about 75°C, and for about 2 weeks at 20°C. It remains in solution and infective at pH 5.0, and can be purified by differential and density-gradient centrifugation. The virus particle is a moderately flexuous rod with a probable length of about 477 mμ and a uniform width of about 15 mμ.

The virus to be described was isolated in Indiana from white clover (*Trifolium repens* L.) showing chlorotic mottle and streaks. Indexing tests indicated that it was different from legume viruses commonly found in this area, and studies (4) showed it to be serologically related to isolates termed as white clover mosaic virus (3, 8, 12, 14).[1] The relation of the present isolate to the unobtainable legume viruses listed in the older literature (9, 10, 11, 16) as white clover mosaic virus or strain is uncertain. This paper describes the host range, properties, purification, and morphology of the virus. Results have been abstracted (1).

**Materials and methods.**—Infected bean (*Phaseolus vulgaris* L. var. Bountiful), since it supports a high concentration of virus, was used as a virus source for purification studies of white clover mosaic virus (WCMV). The upper systemically infected leaves contained about 3 times as much virus as did the primary leaves. The upper leaves were usually frozen and then macerated in an equal weight of water. The expressed sap was centrifuged 10 minutes at 2000 rpm in an Adams Safeguard Centrifuge and the supernatant was adjusted to pH 5.0 with 10% (V/V) acetic acid. After standing ½ hour at 4°C, the material was centrifuged as before and the relatively clear supernatant was further clarified by centrifuging 15 minutes at 10,000 rpm in a Spinco Model L Preparative Ultracentrifuge. The supernatant from this step, either directly or following 1 or 2 cycles of differential angle-head centrifugation, was subjected to density-gradient rate-zonal centrifugation (5) for 2 hours in an SW 25.1 rotor at 24,000 rpm. Two-ml samples were used on columns that had stood overnight at 4°C containing 4, 7, 7, 7 ml of solutions of 10, 20, 30, 40 g sucrose per 100 ml of either 0.01*M* pH 7.0 phosphate buffer or 0.01*M* $Na_2SO_3$. The final temperature of the density-gradient tubes after centrifugation was about 18°C. The visible virus zone as well as the regions immediately above and below were removed from the tubes by bent hypodermic needles attached to syringes. The samples were examined semiquantitatively for infectivity and serological activity, and qualitatively for particle type in the electron microscope.

The infectivity tests, involving either 8 or 12 half-leaf replications per treatment, were made on the primary leaves of Bountiful bean. The development of necrotic lesions was sometimes erratic if the plants were left in the greenhouse at 70–80°F under daylight. For best result, the plants were held 1 day at 90°F under artificial light about 3 or 4 days after inoculation.

The virus used as antigen was prepared by acid clarification followed by two cycles of centrifugation.

[1] After this article had been submitted for publication there appeared an article by Fry, P. R., R. Grogan, and J. W. Lyttleton, 1960, Physical and chemical properties of clover mosaic virus, Phytopathology, 50:175-177. Tests with antiserum and virus kindly provided by Professor R. G. Grogan show clover mosaic virus to be serologically related to white clover mosaic virus.

**Table 1.**—The host range of white clover mosaic virus from Indiana.

| Host plant | Reaction on inoculated leaves | Systemic reaction |
|---|---|---|
| CONVOLVULACEAE | | |
| *Ipomoea purpurea* Lam. vars. Royal Crown and Blue Star | LL[a] | –[b] |
| CUCURBITACEAE | | |
| *Citrullus vulgaris* Schrad. var. Dixie Queen | LL | +[c] |
| *Cucumis sativus* L. vars. National Pickling and Marketeer | LL | – |
| *Cucurbita pepo* L. var. Sugar Pie | LL | – |
| *C. pepo* L. var. *melopepo* Alef. var. Zucchini | LL | – |
| LEGUMINOSAE | | |
| *Cyamopsis tetragonaloba* (L.) Taub. | LL | + |
| *Crotalaria spectabilis* Roth. | LL | + |
| *Glycine max.* (L.) Merr. var. Gibson | LL | S[d] |
| *Medicago sativa* L. var. Ranger | + | + |
| *M. lupulina* L. | + | + |
| *Melilotus alba* Desr. var. Evergreen | + | + |
| *Phaseolus vulgaris* L. vars. Bountiful, Idaho Refugee, Kentucky Wonder, Great Northern, Contender, Pinto, Black Valentine | LL | + |
| *P. lunatus* L. var. Henderson Bush | + | |
| *P. mungo* L. | LL | + |
| *Pisum sativum* L. vars. Pride, Perfected Wales, Alaska, Loyalty, Perfection, Canning King | + | + |
| *Trifolium hybridum* L. | + | + |
| *T. incarnatum* L. | + | + |
| *T. pratense* L. var. Kenland | + | + |
| *T. repens* L. and *T. repens* L. f. *giganteum* Lagr.-Foss | + | + |
| *Vicia faba* L. var. English Broad Windsor | + | + |
| *Vigna sinensis* (Torner) Savi var. Black Eye | LL | + |
| SOLANACEAE | | |
| *Datura innoxia* Mill. | LL | – |
| *D. stramonium* L. | LL | – |
| *Lycopersicon esculentum* L. vars. Pritchard & Purdue 1361 | S | S |
| *Nicotiana sylvestris* Speg. & Comes | LL | – |
| TROPAEOLACEAE | | |
| *Tropaeolum majus* L. var. Glorious Gleam | S | S |

[a] LL designates any type of localized lesion on inoculated leaves
[b] – designates no infection. All of these tissues were indexed on Bountiful bean, with negative results.
[c] + designates any type of infection other than a localized lesion.
[d] S designates a symptomless infection.

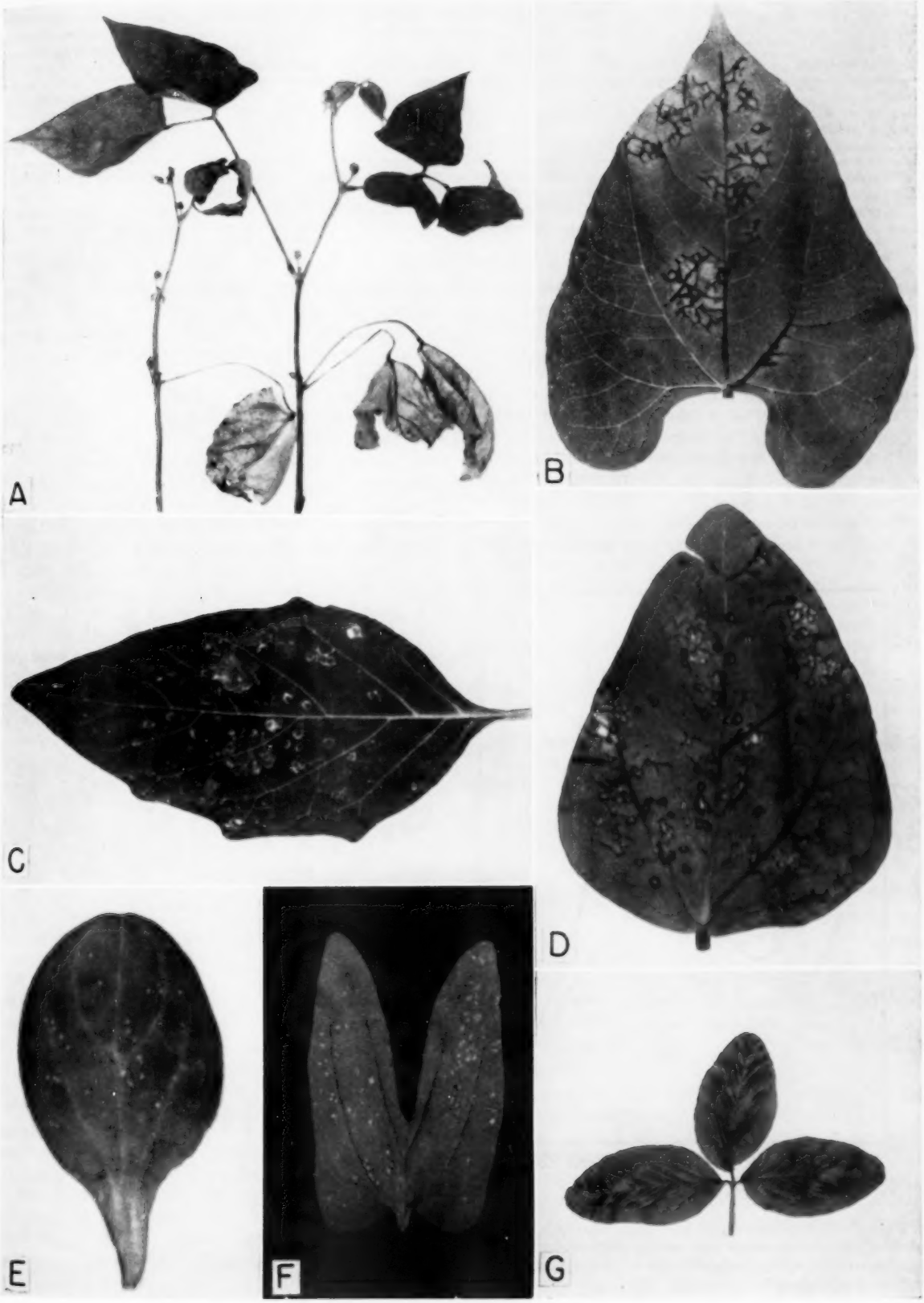
A rabbit, previously bled for normal serum, was injected intravenously 3 times at 4-day intervals with 0.4–0.8 ml of about a 4 mg/ml virus preparation that had been stored by freezing in neutral buffered physiological saline. The rabbit was bled 4 days after the last injection. The antiserum had a titer of 1/512 against virus, and one of 1/8 against healthy plant material as measured by the sensitive interfacial ring-precipitin technique. The standard tube precipitin test showed a titer of 1/128 against virus and <1/4 against healthy plant material. Specific identification of virus or related protein was made by using antiserum dilutions of 1/50 for the tube test and 1/128 for the ring test. Known volumes of the samples removed from density-gradient tubes were dialyzed against buffered saline overnight at 4°C. and 2-fold dilutions were reacted against antiserum in either ring or tube precipitin tests.

Preparations dialyzed against deionized water for 2 days at 4°C and with polystyrene latex spheres added, were sprayed onto collodion-coated grids with nebulizers. The grids were shadowed with chromium and examined in an RCA EMU electron microscope. Particles were measured through a calibrated binocular dissecting microscope.

Ultraviolet measurements were made on a Beckman-DU spectrophotometer. Corrections for scattering were made by the Raleigh rule (13).

**Results and discussion.**—*Transmission and host range.*—The virus was easily transmitted mechanically. Merely rubbing intact bean leaves together transmitted the virus from bean to bean. Tests with *Cuscuta* sp. indicated that dodder transmission is rare, if it occurs at all. In tests with 14 plants, 1 virus transfer may have occurred, but confirmation was prevented by death of the red clover host plant before a second indexing. Bos et al (3) demonstrated dodder transmission of a WCMV isolate serologically similar to ours, whereas Scott and Gold (14) reported none with another isolate (AC-181, ATCC ed. 2) in the same

**Fig. 1.**—Reactions of various hosts to white clover mosaic virus. **A)** Systemic symptoms on *P. vulgaris* var. Bountiful. **B)** Localized reaction on *P. vulgaris* var. Bountiful primary leaf. **C)** Localized reaction on *D. stramonium*. **D)** Localized reaction on *V. sinensis* var. Blackeye primary leaf. **E)** Localized reaction on cotyledon of *C. vulgaris* var. Dixie Queen. **F)** Localized reaction on cotyledon of *I. purpurea* var. Blue Star. **G)** Systemic symptoms on *T. pratense* var. Kenland.


A
B
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E
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**Table 2.**—A comparison of some clarifying methods for white clover mosaic virus from trifoliolate leaves of bean.

| Method | Clarification | Local lesions per 8 half-leaves | Virus zone intensity in density-gradient rate tube |
|---|---|---|---|
| none[a] | slight | 322 | not tested |
| Heated at 40°C for 40 min. at pH 6.0 | good | 201 | moderate[b] |
| Cooled at 4°C for 30 min. at pH 5.0 | good | 292 | strong[b] |
| Chloroform, n-butanol emulsion | good | 16 | none[b] |

[a] Expressed sap from frozen tissue diluted with 2 vols. of water and centrifuged 15 min. at 10,000 rpm in No. 30 rotor. All preparations went through these steps.

[b] Concentrated for ¾ hour at 39,000 rpm in No. 40 rotor, brought up in 0.01*M* $Na_2SO_3$, centrifuged at low speed, and then placed on 0.01*M* $Na_2SO_3$-sucrose columns for rate density-gradient centrifugation for 2 hours at 21,000 rpm in No. SW 25.1 rotor.

serogroup. No soil transmission with bean or seed transmission with *Medicago lupulina* L. could be demonstrated. Extensive trials with *Macrosiphum pisi* Kalt. and *Aphis gossypii* Glover—involving fasting, various infection and test feeding times, and various hosts (all indexed on Bountiful bean)—were negative.

The host-range studies showed that 26 species in 6 families were susceptible to the virus (Table 1). Fig. 1 shows some of the diagnostic symptoms that help differentiate the Indiana WCMV from other legume viruses and other strains.

The symptoms varied with various temperatures. The localized lesions on bean were associated with a later systemic development of symptoms. Single lesions taken from morning glory and transferred to bean produced both types of symptoms, as did single lesions from *Nicotiana sylvestris* Speg. & Comes, and single-lesion and tip-leaf isolates from bean.

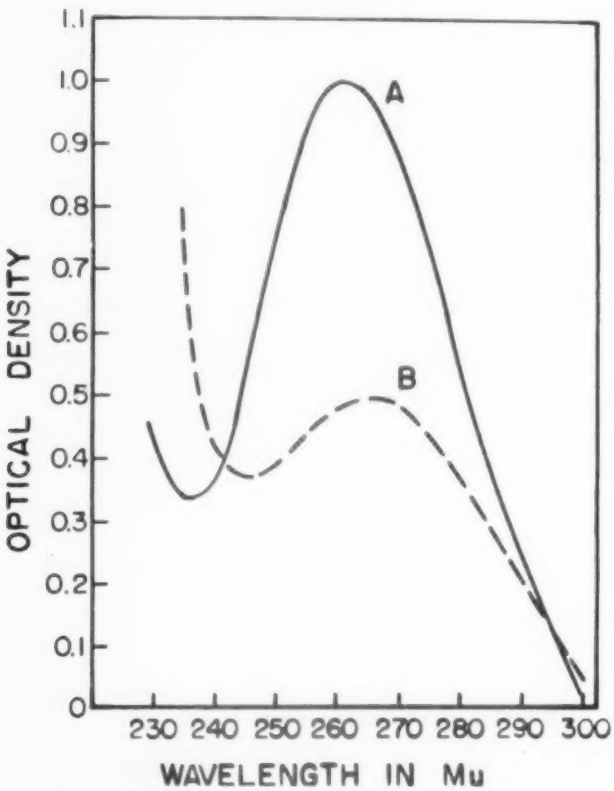



**Fig. 2.**—Ultraviolet light absorption by: **A)** partially purified virus preparation from young tissue before density-gradient centrifugation, and **B)** the same preparation after density-gradient centrifugation.

Twenty-five species in 12 families failed to become infected by the virus. An average of 18 (range 7–30) test plants per species were inoculated. All plants were indexed on Bountiful bean, with negative results. The insusceptible species are: *Amaranthus retroflexus* L.; *A. tricolor* L. var. *splendens* Bailey; *Antirrhinum majus* L. vars. Rust Proof and Rust Proof Maximum Giant Flowered; *Apium graveolens* L. var. *dulce* Pers. vars. Giant Pascal and Easy Blanching; *Beta vulgaris* L. var. Detroit; *Brassica oleraceae* L. var. *capitata* L. var. Danish Ball Head; *Callendula officinalis* L.; *Callistephus chinensis* Nees var. Giant Branching; *Capsicum frutescens* L. var. California Wonder; *Chenopodium amaranticolor* Coste & Reyn.; *Eschscholzia californica* Cham.; *Gomphrena globosa* L.; *Helianthus gigantus* L.; *Matthiola incana* R. Br. var. Bismark Large Flowering; *Nicotiana glutinosa* L.; *N. tabacum* L. vars. Wis. Hav. 38 and 423; *Petunia hybrida* Vilm. var. Celestial Rose; *Phaseolus coccineus* L.; *Ricinus communis* L. var. Zanzibar; *Sida spinosa* L.; *Solanum carolensis* L.; *S. melongena* L. var. *esculentum* Nees, var. Black Beauty; *S. nigrum* L.; *Tetragonia expansa* Murr.; *Zinnia elegans* Jacq. var. On Parade.

*Relationship of 3 isolates of the virus.*—Bos et al (4) showed that isolates of WCMV from this country, Canada, Holland, and Germany were serologically related. Cross-protection tests involving Bos' and Scott and Gold's isolates as the established virus and with the Indiana isolate as the challenge on bean supported the serological data. In the cross-protection tests, Bos' isolate gave partial protection to the local lesion produced by the Indiana isolate on the primary leaves of bean and complete protection against the systemic symptoms consisting of death of the primary leaves, necrotic stem lesions, and severe necrosis and inward curling of the trifoliolate leaves. Reciprocal cross-absorption tests showed no differences between these two isolates (4). Scott and Gold's isolate, which is serologically related to but distinguishable from the Indiana strain (4), gave no protection against the local reactions, but complete protection against the systemic ones.

**Table 3.**—Relationships of zone, infectivity, serological reaction, and particle type.

| Fraction | Distance from meniscus (mm)[a] | Relative infectivities[b] | Serological reaction[c] | Predominant particle length (mμ)[d] |
|---|---|---|---|---|
| Above-zone | 18.2–21.2 | 8.8±8.7 | + | < 450 |
| Visible-zone | 22.5–24.2 | 89.5±14.5 | + | > 450 |
| Below-zone | 24.9–27.6 | 91.9±12.0 | + | > 450 |

[a] An attempt was made to remove the samples from the same relative position in each of 14 experiments. All density-gradient centrifugations run for 2 hours at 24,000 rpm in an SW 25.1 rotor.
[b] The data are the average of 14 experiments. All experiments were given the same weight, and variation is expressed as standard deviation.
[c] Determinations made in 12 experiments.
[d] Fractions from 6 experiments examined in the electron microscope.

The isolates could be conveniently distinguished on several hosts. Only Scott and Gold's isolate produced local lesions on *G. globosa* (Dr. A. H. Gold suggested this host). Only the Indiana isolate produced local lesions on morning glory (Fig. 1-F), *D. inoxia*, and *D. stramonium* (Fig. 1-C). Bos' isolate alone caused an obvious systemic green mottle in soybean. The Indiana isolate was much more severe on bean (Fig. 1-A, B) and cowpea (Fig. 1-D) than the other two isolates. All isolates produced local lesions on watermelon cotyledons (Fig. 1-E) and systemic streaking on red clover (Fig. 1-G).

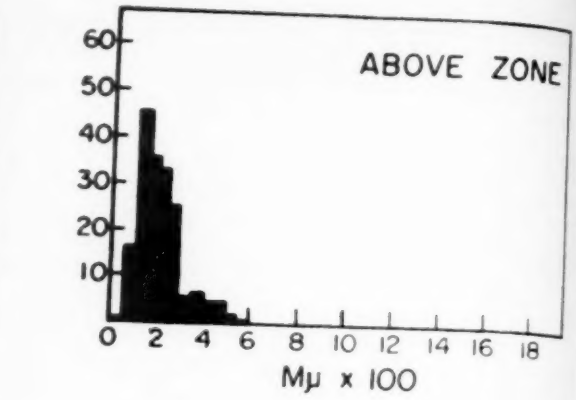
*Physical properties.*—Each test was run from macerated systemically infected bean tissue (ca. pH 6.3) at least 4 times. The results varied. The minimum and maximum values where infection was last found follows: dilution end-point, $10^{-5}$–$10^{-6}$; longevity *in vitro* at 20°C, 9–14 days; thermal inactivation, 75–80°C for 10 minutes. The local and systemic symptoms usually accompanied each other. These values do not agree with any of those listed for the originally described WCMV isolates (11, 16) or, except for the dilution endpoint data, with those of Bos et al (3) and Quantz (12).

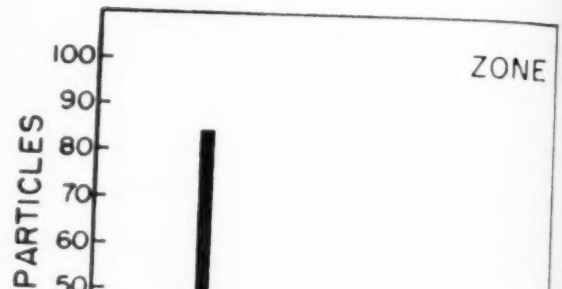
*Description of preparations.*—The initial clarification method by acidification to pH 5.0 resulted in little or no loss of virus activity from that of the control material (Table 2). In comparison, about ⅓ of the virus was inactivated at pH 6.0 at 40°C for 40 minutes, and the virus was lost when extracted by the emulsion method (15). After 1 or 2 cycles of differential ultracentrifugation (the virus could also be precipitated with 0.5 sat. $(NH_4)_2SO_4$) of clarified material, preparations exhibited an ultraviolet light absorption curve typical of nucleoprotein (Fig. 2). Comparable control material showed only slight absorption. When the concentrated virus preparations were subjected to density-gradient centrifugation and again measured in ultraviolet light, the absorption curve indicated a decrease in nucleic acid (Fig. 2). This decrease resulted from the separation from virus of what was probably ribosomal contamination that sedimented at a rate slower than did the virus. This slow-moving visible zone was usually present in preparations made from young upper leaves, but not from older leaves. A suitable detergent (6) would probably increase the efficiency of separation. The absorption data show that the virus is a nucleoprotein and that it has a nucleic acid content of about 8% ($E\frac{260\ m\mu}{280\ m\mu}=1.33$).

The depth of the top of the visible infectious zone from the meniscus of density-gradient tubes averaged 22.5 mm after 2 hours at 24,000 rpm at a final temperature of 18°C. The zone was sharp and easily identifiable in clarified preparations and in those which had first been concentrated and suitably diluted. The respective depths after 1 and 3 hours of centrifugation were about 13.2 mm and 30.0 mm. The isolates of Bos and Scott and Gold behaved similarly. The virus sedimented much more slowly than bean yellow mosaic virus and red clover vein mosaic virus, both of which reached depths of about 30 mm below the meniscus after 2 hours of centrifugation under the same conditions. An analytical centrifugation observation kindly made by Dr. J. Kelley, of about a 0.5% solution of WCMV in 0.01*M* pH 7.0 phosphate buffer at 22.6°C, showed an $S_{obs} = 111$.

*Relationship of infectivity and particle type.*—Table 3 shows that, in contrast to the region immediately above the visible zone in density-gradient tubes, the visible zone and the region below it were highly infectious. Further down the tube (around 30 mm), infectivity was low. Visual observations indicated that there was more virus protein in the visible zone than in the regions immediately adjacent. The serological reactivities in these areas varied, but generally confirmed the visual observations, the average ratio of above-zone: zone:below-zone being respectively about 1:3:2. These data cannot be used to express specific infectivities, however, because it cannot be assumed that different particle types have equal serological reactivities. It can only be said that the protein in the different areas is serologically related.

Electron micrographs of particles from the three fractions and accompanying histograms are in Fig. 3. In the region above the visible zone in density-gradient tubes, the predominant particle length was between 100 and 300 mμ. These short particles are probably not preparative artifacts. The infectivity data (Table 2) show that the clarification procedure was gentle. Clarified preparations placed directly on density-gradient columns, containing either 0.01*M* $Na_2SO_3$ or 0.01*M* phosphate buffer (pH 7.0) as a solvent, showed the presence of short particles. They were present regard-



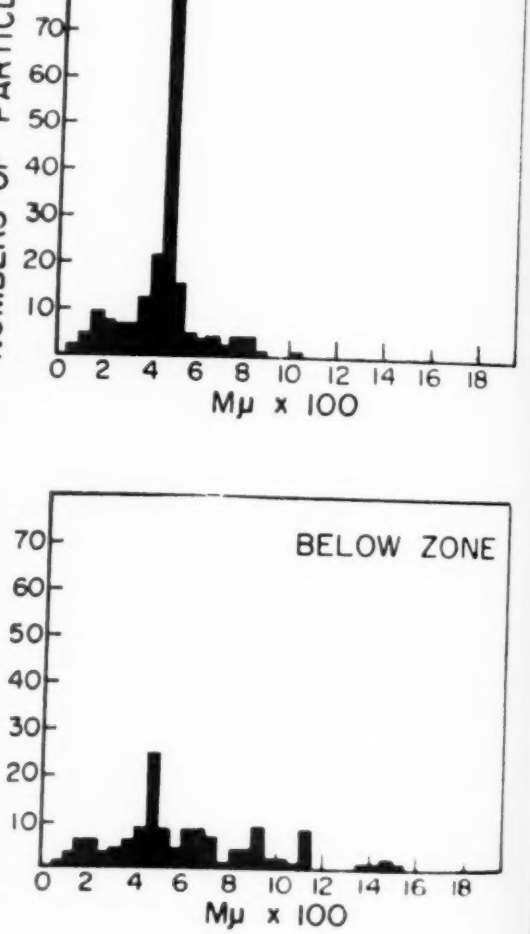




**Fig. 3.**—Electron micrographs (X 30,700) and histograms of particles removed from above the visible zone, in the visible zone, and below the visible zone. The small spherical particles in the topmost micrograph are possibly ribosomes. Note the long threadlike projections from the particles shown in the inset (X 45,900).

less of the method of application (spraying or dropping) to the grids. The figure of unfractionated WCMV of Bos et al (3) contains many short particles. Uninfectious short particles have also been found with tobacco mosaic (2) and wheat streak mosaic (7) viruses. The predominant particle length in the infectious visible zone region was 450–500 mμ. The average length of the particles in the modal distribution was 477 mμ. This length agrees with those published elsewhere (3, 8, 14). These rods presumably are virus particles. A quantitative study relating particle numbers of this class to some specific infectivity level is still to be done, however. The infectivity of the above-zone region was probably due to small numbers of these particles. Particles from the zone region were occasionally seen with threadlike projections that may represent nucleic acid. This interpretation was not tested by ribonuclease experiments. The generally long rods found in the region immediately below the visible zone seem to be a least as infectious as those found in that zone. These data probably resulted partially from sampling admixtures of particles from above the visible zone with those in the zone and, again, particles of the visible zone with particles from below the zone. All particles had a uniform width of about 15 mμ. No rods were seen in control preparations.

The serological data showed that the different particle types resulted from infection with one virus. Another experiment in which a series of ½-log dilutions were made from the visible zone and below-zone fractions supports this conclusion. The inoculum from the visible zone infected 1 of 16 beans at $10^{-2}$, whereas that from the below-zone region infected 2 of 19 plants at $10^{-2.5}$. Only one lesion could be seen on each of the plants, and the symptoms on the plants were the same. Subinoculations to other species produced the same symptoms. Preparations were made from beans infected with the different inocula and with a mixture of inocula. No differences could be seen in the preparations when they were examined in the centrifuge.—Department of Botany and Plant Pathology, Purdue University, Lafayette, Indiana.

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# A SERIAL DILUTION END-POINT METHOD FOR ESTIMATING DISEASE POTENTIALS OF CITRUS PHYTOPHTHORAS IN SOIL

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The author thanks Albert Amorteguy, who assisted in most of the disease potential tests.

SUMMARY

A quantitative method was devised for studying the distribution of *Phytophthora citrophthora* and *P. parasitica* in the soil and factors influencing their disease potentials *in situ*. Lemon fruit was used as host to detect the fungi in infested soils serially diluted with sterile soil, placed in paper cups, and incubated under standardized conditions. Soils with different levels of *Phytophthora* infestation produced different end-points of fruit infection in the dilution series. From these end-points, comparative levels of disease potential were estimated. Disease Potential Indices of 0–32 were obtained from tests with 140 soils. Results from later tests, performed to evaluate the method, indicated that the disease potential data obtained with this method were sufficiently accurate and reproducible.

*Phytophthora citrophthora* (R. E. Sm. & E. H. Sm.) Leonian and *P. parasitica* Dast. are the 2 main causal fungi of fibrous root rot of citrus (8, 11). Recently, studies have been initiated on the ecological factors affecting the disease potentials of these fungi in the soil. In these studies, the need for a quantitative procedure for estimating their disease potentials is obvious; none of the conventional methods are applicable to *Phytophthora* spp., which do not develop on agar

media of soil dilution plates (e.g., 3, 13, 14, 24). Information is lacking on *Phytophthora* spp. obtained with other quantitative methods (1, 4, 9, 15, 21, 22). Even if any of these methods were successful, one would still have to isolate Phytophthoras in pure culture for identification and for tests of their pathogenicity on citrus. The per cent infection method (6, 23), assessing disease on the basis of percentage infection received by a large number of susceptible seedlings planted in a given amount of infested soil in the greenhouse, did not work for the citrus Phytophthoras, probably because of the nature of their infective units, the swarming zoospores. Under favorable conditions, infection was 100% even with a soil containing only a small amount of the fungus. The long duration of the experiment also makes these methods undesirable. The lemon trap method (10) proved to be an excellent method for isolating citrus Phytophthoras, but is only of a qualitative nature. While it is capable of detecting their presence in the soil, it does not give an indication of the degree of Phytophthora infestation in the soil sample tested.

Because the above methods, many tested by the author, were infeasible for these fungi, a completely different approach was used. By adapting the principles of serial dilutions such as in fungicide or antibiotic bioassays, a "serial dilution end-point" method was devised. The rationale of the method was that serial dilutions of any infested soil (original, 1-in-2, 1-in-4, 1-in-8, etc.) with sterile soil as the diluent would eventually reduce the disease potential to zero. Thus, a given soil containing different amounts of *Phytophthora* would produce different end-points of brown rot infection in the dilution series. From these end-points, comparative levels of *Phytophthora* population could be estimated. It somewhat resembles the dilution end-point method used in a virus inactivation study, and the various dilution-frequency methods for quantitative estimation of algae, bacteria, and other soil microorganisms (2, 5, 12). It differs from the others, however, in that the diluent is sterile soil instead of water, and that extensive replication is not used for calculating the most probable number.

**Preliminary trials.**—Numerous preliminary experiments with several field soils naturally infested with citrus *Phytophthora* spp. indicated that, in most cases,

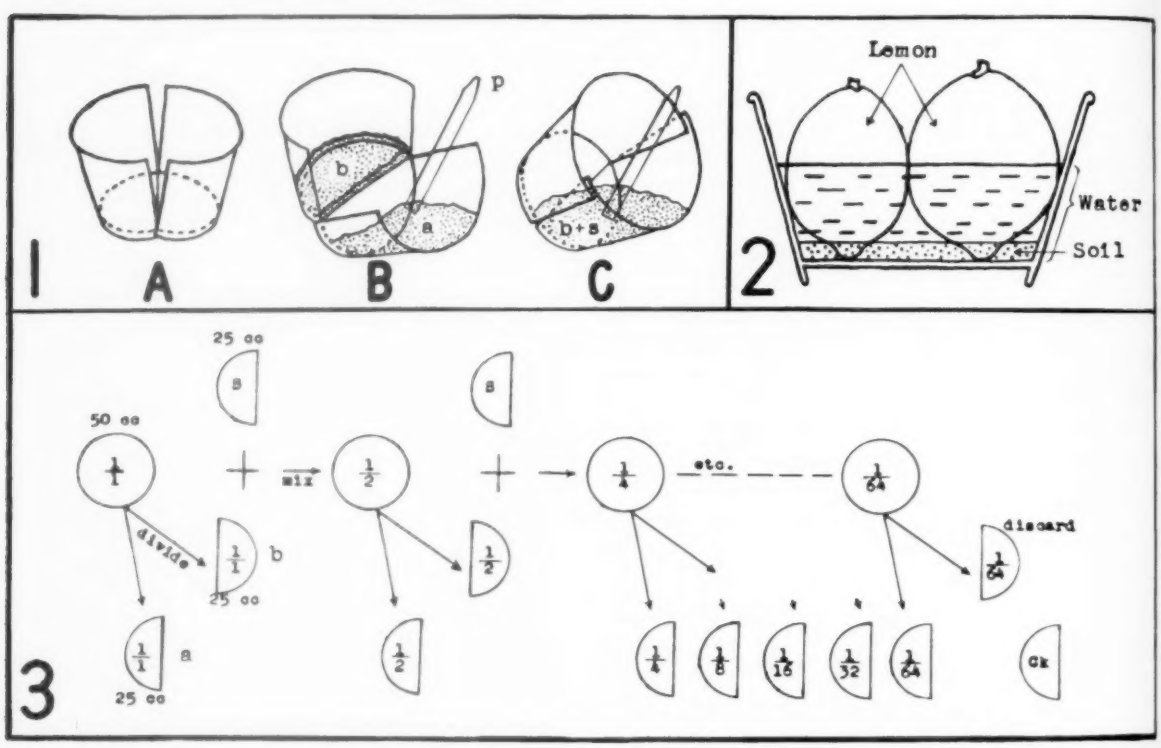


**Fig. 1-3.—1) A)** the "split-cup" made from the 16-oz. paper cup by cutting the sides to form 2 connected, movable, equal halves. A different cup is used for each soil sample. **B), C)** The split-cup shown in positions as a soil divider and a soil mixer, respectively. The 50 cc of soil after having been leveled at the cup bottom are divided into 2 equal halves as shown in **B.** One-half of the soil (a) is removed with the aid of a pot label stick (p) into a given dilution cup for the lemon trap test. To the remaining half (b) is then added 25 cc of sterile soil (s) and mixed thoroughly, as shown in **C,** to provide 50 cc of soil (b+s) of the next higher dilution. The mixing is done in the split-cup with a pot label stick by a circular motion while the cup, with portions of the movable halves overlapping, is held at an angle. Efforts are made by scraping with the pot label to avoid the adherence of wet soil particles on the cup walls. **2)** The lemon trap test with each dilution cup, showing the positions of 2 lemon fruits, 150 cc of water, and 25 cc of soil in the cup. **3)** Schematic diagram of the procedures involved in the dividing and the mixing of the soil in the serial dilution end-point method. The 8 semicircles in the bottom row, each representing 25 cc of soil, constitute a single replicate dilution series.

25 cc of the soil and 150 cc of copper-free water in a 1-pint wide-mouthed waxed paper cup in the routine lemon trap test was sufficient to induce a number of *Phytophthora* lesions on lemon fruits at 25±3°C. Most brown rot lesions developed and became recognizable in 5 days under these conditions. More than one lemon fruit was needed per cup to avoid disease escape resulting from immaturity or other defects of some fruits. The cup preferred was the 16-oz. Dixie container, No. 2186-ST, Dixie Cup Company, Easton, Pa. Also satisfactory are cups of the same size and quality by other manufacturers.

A preliminary experiment indicated that dilutions of the original field soils with a steam- or autoclave-sterilized soil, though quantitatively affecting the population, did not appear to impede the ability of the fungi to incite lesions on lemon fruits. In the 5 soils tested in 6 different trials, the end-points of brown rot infection in the serial dilutions were different for these soils. Some were at 1-in-1 dilution (the original); others were at 1-in-4 or 1-in-8 dilutions. In one soil, the end point was at 1-in-32 dilution. The highest dilution in which brown rot lesions were ever found in any soil was 1-in-32. Therefore, it was believed unnecessary to dilute the soil further than the 1-in-64 dilution in future tests. (The 25 cc of the 1-in-64 diluted soil would theoretically contain only about 0.4 cc of the original soil.)

From information obtained in the preliminary experiments, a standardized method was evolved. That work has been briefly reported (16).

**Materials and methods.**—Five hundred to 800 cc of a soil sample to be tested was pulverized by hand to fine particles (2 mm or less with most soils) and mixed thoroughly. Fifty cc of the soil, measured with a 50-cc beaker, was placed in a "split-cup" (Fig. 1-A) and divided into 2 equal portions (Fig. 1-B). One-half was put into a paper cup and labeled "1/1"; 25 cc of an autoclaved soil (about pH 7) was added to the remaining half and mixed thoroughly with a clean pot label stick (Fig. 1-C) to provide a 1-in-2 dilution. This procedure was repeated to give the 7 dilutions of 1/1, 1/2, 1/4, 1/8, 1/16, 1/32, and 1/64 (Fig. 3). Another 50 cc of the original soil was treated in the same manner to provide a replicate. Two cups, each containing 25 cc of the sterile soil, were used as controls. Deionized water (150 cc) was added to each cup, and 2 lemon fruits (*Citrus limonia* Osbeck, var. Eureka or Lisbon) at the ripe or near-ripe stage were put into each cup with the peduncle ends up and the distal halves immersed in the mud-water mixture (Fig. 2). All cups were incubated at about 25°C. At 6 days, qualitative data were recorded (either positive or negative infection reading) for brown rot lesions on the fruits in each cup. One or more brown rot lesions on either or both of the 2 fruits in a cup gave a positive infection reading for that dilution cup. The Disease Potential Index (DPI) of a given soil was defined as the reciprocal of the highest of the dilutions that yielded brown rot lesions on the lemon fruit under the test conditions. When the 2 replicate dilution series gave dissimilar results, the average of the 2 Indices was made the DPI of the soil tested. Soil with an index of 16 is considered to have a disease potential about 8 times as high as that of a soil with an index of 2.

If, in the dilution series, there was a negative fruit infection followed by a positive, such as ++−+−−−, the DPI was taken as the reciprocal of the dilution

**Table 1.**—Phytophthora disease-potential tests with 17 representative soils.

| Test no.[a] | Soil no.[b] | Dilution 1/1 | 1/2 | 1/4 | 1/8 | 1/16 | 1/32 | 1/64 | DPI[e] |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 1 | +[d] | + | + | + | + | −[d] | − | 16 |
| | | + | + | + | + | + | + | − | 32 |
| 4 | 5 | + | − | − | − | − | | | 1 |
| | | + | − | − | − | − | | | 1 |
| 13 | 21[c] | + | + | + | − | − | − | − | 4 |
| | | + | + | + | − | − | − | − | 4 |
| | 22[c] | + | + | + | − | − | − | − | 4 |
| | | + | + | − | − | − | − | − | 2 |
| 21 | 59 | − | − | − | − | − | − | − | 0 |
| | | − | − | − | − | − | − | − | 0 |
| | 60 | + | + | − | − | − | − | − | 2 |
| | | + | − | − | − | − | − | − | 1 |
| | 61 | + | + | + | + | − | − | − | 8 |
| | | + | + | + | + | − | − | − | 8 |
| | 62 | + | + | + | + | − | − | − | 8 |
| | | + | + | + | + | − | − | − | 8 |
| | 63 | + | + | + | + | − | − | − | 8 |
| | | + | + | + | + | − | − | − | 8 |
| 27 | 92 | + | + | + | + | − | − | − | 8 |
| | | + | + | + | + | − | − | − | 8 |
| | 93 | + | + | + | + | + | − | − | 16 |
| | | + | + | + | + | + | − | − | 16 |
| | 98[c] | + | − | − | − | − | − | | 1 |
| | | + | − | − | − | − | − | | 1 |
| | 100 | + | + | − | − | − | − | − | 2 |
| | | + | + | − | − | − | − | − | 2 |
| | 101 | + | + | + | + | + | + | − | 32 |
| | | + | + | + | + | + | + | − | 32 |
| 31 | 138[c] | + | + | + | + | + | + | − | 32 |
| | | + | + | + | + | + | + | − | 32 |
| | 139 | + | + | + | + | + | − | − | 16 |
| | | + | + | + | + | + | + | − | 32 |
| | 140[c] | + | + | + | − | − | − | − | 4 |
| | | + | + | + | + | − | − | − | 8 |

[a] For each test, 2 or 3 replicate cups of control (sterilized) soil were used, except for test no. 21, a test on effects of soil pH. In that, 2 replicate cups of control soil were used for each of the 5 soils of 5 different pH. All control soils tested negative, resulting in no lesions.

[b] Each soil was tested in 2 replicates, a and b, with results respectively indicated in first and second lines.

[c] *Phytophthora* sp. was *P. citrophthora*; all others were *P. parasitica*.

[d] The "+" sign indicates the presence of 1 or more brown rot lesions on either or both of the 2 lemon fruits in the cup. The "−" sign indicates the absence of lesions on the fruits.

[e] DPI = Disease Potential Index, defined as the reciprocal of the highest of the dilutions that yielded brown rot lesions on the lemon fruit under the test conditions.

**Table 2.**—Phytophthora disease-potential tests using more than 2 replicate dilution series.

| Test no. and soil[a] | Dilution 1/1 | 1/2 | 1/4 | 1/8 | 1/16 | 1/32 | 1/64 | DPI[d] |
|---|---|---|---|---|---|---|---|---|
| 1—T1 | +[c] | + | + | + | + | —[c] | — | 16 |
| | + | + | + | + | — | — | — | 8 |
| | + | + | + | + | — | — | — | 8 |
| 2—CCD | + | + | + | — | — | — | — | 4 |
| | + | + | + | — | — | — | — | 4 |
| | + | + | + | — | — | — | — | 4 |
| 3—LDS[b] | + | + | + | + | — | — | — | 8 |
| | + | + | + | + | — | — | — | 8 |
| | + | + | + | + | — | — | — | 8 |
| 4—T2 | + | + | + | — | — | — | — | 4 |
| | + | + | + | — | — | — | — | 4 |
| | + | + | + | — | — | — | — | 4 |
| | + | + | + | — | — | — | — | 4 |
| | + | + | + | + | — | — | — | 8 |

[a] For each test, 2 replicate cups of control (sterilized) soil were used. All control soils tested negative, resulting in no lesions. Three replicate dilution series were used in tests 1, 2, and 3; and 5 in test 4; with results respectively indicated in separate lines.

[b] *Phytophthora* sp. was *P. citrophthora*; all others were *P. parasitica*.

[c] The "+" sign indicates the presence of 1 or more brown rot lesions on either or both of the 2 lemon fruits in the cup. The "—" sign indicates the absence of lesions on the fruits.

[d] DPI = Disease Potential Index, defined as the reciprocal of the highest of the dilutions that yielded brown rot lesions on the lemon fruit under the test conditions.

preceding the highest positive dilution. In this case, the DPI is 4.

When soils known to contain only small amounts of *Phytophthora* are tested, there will be no need to extend the dilutions so high. Similarly, fewer (2 or 3) serial dilutions are necessary for a soil used as the control in an experiment where artificial soil infestation is used.

Brown rot lesions on lemon fruits infected by either *P. citrophthora* or *P. parasitica* have a characteristic appearance and odor, and are not easily confused with lesions produced by other organisms. In tests with soils artificially infested with a known *Phytophthora* species, brown rot infection on test fruits could be determined without resorting to isolation of the fungus from the lesions. When field soils infested with *Phytophthora* of unknown species were used, the fungus was isolated for identification simply by plating the seeds of the infected lemon aseptically on a suitable agar medium (e.g., potato-dextrose agar, corn-meal agar). Pure culture of the fungus was usually obtained.

**Results.**—The method described was used in 31 separate tests to determine the disease potentials of citrus *Phytophthora* spp. in 140 soils—about one-third naturally infested field soils and the remainder artificially infested soils used in many laboratory and greenhouse experiments that had undergone various treatments, e.g., effects of soil pH, of soil microbial factors, of soil moisture, and of soil fungicides. Because of high summer temperatures in the greenhouse, *P. parasitica*, which has a higher optimal temperature requirement than *P. citrophthora*, was used as the test organism in most of the artificially infested experimental soils. The DPI of *Phytophthora* spp. produced by these soils ranged from zero to 32. The results of some representative tests are given in Table 1.

**Evaluation of the method.**—A number of tests were made to evaluate the accuracy and reproducibility of the serial dilution end-point method.

*More than 2 replicate dilution series.*—With about one-fifth of the 140 soils tested, the 2 replicate dilution series produced end-points at 2 different, though consecutive, dilutions (Table 1). Consequently, the DPI for the 2 replicates differed, and an average index was used to indicate the disease potential of the soil tested. Experiments were carried out to determine the number of replicate series required for reliable results. Four tests were made, using 3 or 5 replications. The data (Table 2) indicate that the method produced surprisingly uniform results. In tests 2 and 3, all 3 replicate series yielded the same end-point, at 1-in-4 and 1-in-8, respectively. In tests 1 and 4, one of the replicate series produced an end-point different from the others, giving the replicate a higher index value than the rest. Nevertheless, 2 replicate dilution series for each soil sample would be considered adequate in routine tests.

*Soils containing known relative amounts of inoculum.*—Various soils containing different known relative amounts of *Phytophthora* were prepared. The DP test was performed with each of the soils to see whether the DPI obtained would reveal *Phytophthora* concentrations accurately. Three field soils naturally infested with *P. parasitica* were used, from which various soils containing different known amounts of the original soil were prepared by using steam-sterilized soil of the same source as the diluent. The results (Table 3) showed that the DPI of *P. parasitica* were correlated with the relative concentrations of infested soils in these dilutions.

*Correlation between the estimated disease potential and root damage.*—The same 3 soils used in test 3 of Table 3 were used in a greenhouse pot experiment to compare the estimated DPI of *P. parasitica* with the root damage caused by the fungus in these soils. The same soil after steam sterilization was used as the control. A 2-month-old seedling of sweet orange (*Citrus sinensis* Osbeck, var. Homosassa) was transplanted to each 5-in. clay pot containing a uniform amount of each of the 4 soils. Each treatment was replicated 8 times in randomized blocks on the greenhouse bench. The periodic waterlogging method (19) was used in watering the plants. Roots were harvested at 1 month, and root damage was assessed by the healthy root tip count method (19). The *Phytophthora* DPI for each soil was related to the degree of root damage (Table 4).

*Tests with the same soil at 3 different moisture levels.*—It is known that soil moisture greatly influences the activity, including sporangium production, of *Phytophthora* spp. in the soil. Experiments by Dimitman (7)

**Table 3.**—Results of disease-potential tests of soils containing known relative concentrations of *Phytophthora parasitica*.

| Test no. and soil[a] | *Phytophthora* conc.[b] | Dilution 1/1 | 1/2 | 1/4 | 1/8 | 1/16 | 1/32 | 1/64 | DPI[d] |
|---|---|---|---|---|---|---|---|---|---|
| 1—LDS | 1 in 1 | +[c] | + | + | + | −[c] | − | − | 8 |
| | | + | + | + | + | − | − | − | 8 |
| | 1 in 3 | + | + | − | − | − | − | | 2 |
| | | + | + | + | − | − | − | | 4 |
| | 1 in 9 | + | − | − | − | − | | | 1 |
| | | + | − | − | − | − | | | 1 |
| 2—BC | 1 in 1 | + | + | + | + | + | + | − | 32 |
| | | + | + | + | + | + | + | − | 32 |
| | 1 in 5 | + | + | + | + | − | − | | 8 |
| | | + | + | + | + | − | − | | 8 |
| | 1 in 25 | + | + | − | − | | | | 2 |
| | | + | + | − | − | | | | 2 |
| 3—T1 | 1 in 1 | + | + | + | + | − | − | − | 8 |
| | | + | + | + | + | − | − | − | 8 |
| | 1 in 4 | + | + | − | − | − | − | | 2 |
| | | + | + | − | − | − | − | | 2 |
| | 1 in 16 | − | − | − | − | | | | 0 |
| | | + | − | − | − | | | | 1 |

[a] For each test, 2 replicate cups of control (sterilized) soil were used. All control soils tested negative, resulting in no lesions.

[b] 1 in 1 = the original soil; 1 in 3 = one portion of the original soil was mixed with 2 portions of the sterilized soil; 1 in 9 = one portion of the "1 in 3" soil was further mixed with 2 portions of the sterilized soil. Soils in tests 2 and 3 were prepared in the similar manner. Each of the 9 soils was tested in 2 replicates, a and b, with results respectively indicated in first and second lines.

[c] The "+" sign indicates the presence of 1 or more brown rot lesions on either or both of the 2 lemon fruits in the cup. The "−" sign indicates the absence of lesions on the fruits.

[d] DPI = Disease Potential Index, defined as the reciprocal of the highest of the dilutions that yielded brown rot lesions on the lemon fruit under the test conditions.

showed that mycelia of citrus Phytophthoras were generally incapable of infecting lemon fruits, and that zoospores were mainly responsible for fruit infection. It was thought that the DPI of the fungus in a soil might also be influenced by the moisture content of the soil at the time the test was made, that a wet soil might produce a higher DPI than the same soil when it was drier, and that sporangia or zoospores present in an excessively wet soil might influence the test by producing a higher index. A test was therefore carried out in an attempt to answer these questions.

A uniformly mixed field soil, naturally infested with *P. parasitica* and possessing a DPI of 8 (soil 1 in Table 4) was used as the test material. About 300 cc (327–333 g) of soil was placed in each of 3 porcelain pans—to a depth of about 1.3 cm. Pans A, B, and C respectively received 0, 80, and 130 ml of water. Pan A was covered with aluminum foil to prevent moisture loss. All pans were incubated at about 25°C. At the end of 2 days, the previously waterlogged soil in pan B had become much drier, whereas the soil in pan C was still waterlogged, with a considerable amount of free water. Calculations from the wet and dry weights revealed the per cent water content, by weight, of soils A, B, and C to be, respectively, 3.3, 15.5, and 20.3. After thorough remixing, each soil was weighed out in one-sixth aliquots (equivalent to 50 cc of soil of original moisture content).

In addition to a routine DP test for each of the 3 soils (see Test II in Table 5), other tests were performed in an attempt to study the nature of sporangium and zoospore production involved in the mud-water. In one test (Test I in Table 5), 2 lemons were placed in each cup of mud-water in each dilution series of the 3 soils 20 minutes after water was added. They were removed after about 10 minutes and incubated individually in enclosed polyethylene bags. The high relative humidity inside the bags was maintained by enclosing in each bag a cotton ball saturated with sterile distilled water. The bags were incubated at 25°C and brown rot lesions on the fruits were recorded at 6 days. For another test (Test III in Table 5), a separate dilution series was made for each of the 3 soils. Immediately after water was added to the cups, 2 lemon fruits were placed in the mud-water and allowed to incubate at 25°C. Twelve hours later, they were taken out and rinsed under tap water to remove adhering soil particles or possible mycelia from the fruit surface, and further incubated individually in humid polyethylene bags. Another 2 lemon fruits were placed in the mud-water of each cup immediately after the first 2 fruits were taken out. They were treated identically after a 12-hour incubation period in the cups. The procedure was repeated 8 times—at each of the 8 consecutive 12-hour periods. Lesions on the fruits were recorded twice for each of the 24 groups of fruits: once at 6 days after water was added to the cup, and again at 6 days after the fruits were introduced into the mud-water.

The results are in Table 5. The following observations and interpretations were made:

1) The 3 soils, which possessed the same initial amount of *P. parasitica* but were of contrasting moisture levels, produced identical DPI (Test II in Table 5). It is therefore likely that the moisture content of

**Table 4.**—Disease Potential Indices vs. healthy root tip counts.

| Soil | *Phytophthora parasitica* conc. | No. healthy root tips per seedling[b] (av. of 8) | DPI[c] (av. of 2) |
|---|---|---|---|
| 1) Original, infested | 16 × C[a] | 1.5 | 8 |
| 2) ¼ original | 4 × C | 9.9 | 2 |
| 3) $\frac{1}{16}$ original | 1 × C | 29.0 | 0.5 |
| 4) Sterilized (control) | 0 | 58.4 | 0 |

[a] C = the concentration of *P. parasitica* in soil 3.

[b] Linear relationship significant at 5% level.

[c] DPI = Disease Potential Index, defined as the reciprocal of the highest of the dilutions that yielded brown rot lesions on the lemon fruit under the test conditions.

**Table 5.**—Results of disease-potential tests with the same soil at 3 different moisture levels.

| Periods (hours): from water added to lemon added—incubation of lemon in cup | Soil[a] | Results read at: 6 days after water added; Days after lemon introduced | "DPI"[b] | 6 days after lemon introduced; Days after water added | "DPI" |
|---|---|---|---|---|---|
| Test, I, 1/3—1/6 | A | 6 | [c] | 6 | 2 |
| | B | | | | 2 |
| | C | | | | 1 |
| Test II, 1/2—144 | A | 6 | [c] | 6 | 8 |
| | B | | | | 8 |
| | C | | | | 8 |
| Test III, 0—12 | A | 6 | 2 | 6 | 2 |
| | B | | 4 | | 4 |
| | C | | 4 | | 4 |
| 12—12 | A | 5.5 | 4 | 6.5 | 4 |
| | B | | 4 | | 4 |
| | C | | 8 | | 8 |
| 24—12 | A | 5 | 4 | 7 | 4 |
| | B | | 4 | | 4 |
| | C | | 2 | | 2 |
| 36—12 | A | 4.5 | 2 | 7.5 | 2 |
| | B | | 8 | | 8 |
| | C | | 8 | | 8 |
| 48—12 | A | 4 | 8 | 8 | 8 |
| | B | | 8 | | 8 |
| | C | | 4 | | 4 |
| 60—12 | A | 3.5 | 1 | 8.5 | 1 |
| | B | | 4 | | 4 |
| | C | | 0 | | 1 |
| 72—12 | A | 3 | 1 | 9 | 1 |
| | B | | 1 | | 2 |
| | C | | 0 | | 1 |
| 84—12 | A | 2.5 | 0 | 9.5 | 1 |
| | B | | 0 | | 1 |
| | C | | 0 | | 1 |

[a] The % water, by weight, in soils A, B, and C, was respectively 3.3, 15.5, and 20.3 at the start of the tests.

[b] Since, except in Test II, the lemons were incubated in polyethylene bags, the test conditions were not the same as those used in the routine DP tests. Furthermore, the fruit infections in these tests were not necessarily read at 6 days. Therefore, the "DPI" in this table were *modified* DPI, and were not the same as that defined in "Materials and methods" in the text.

[c] Since the lemons were introduced to the water 20–30 minutes after water was added to the cups, the infection readings at 6 days after water was added were the same as the readings in the column to the right, readings at 6 days after lemons were introduced.

a soil tested, though probably influencing the amount of infective units (the zoospores) in the soil at test time, would have little influence on the DPI given by the test at 6 days. In Test I, where lemons were removed 30 minutes after water was added and incubated outside the cup, brown rot lesions were found on some of the fruits 6 days later, indicating the presence of sporangia and/or zoospores in the mud-water during the first 30 minutes. Lesions were never found, however, on any lemon fruits in dilution cups that were beyond the 1-in-2 dilutions in any of the 3 soils in Test I, which was performed with the cups of the same dilution series that were later used for Test II. Since the DPI was much higher for all 3 soils in Test II, all fruits that contracted brown rot in the cups of higher dilutions (1/4 and 1/8) at the end of 6 days must have been infected by the zoospores that were not present during the first 30 minutes of the test.

2) The hypothesis that zoospores produced after addition of water to the cups were largely responsible for infection on the lemon fruits during the test was further supported by the results of Test III. These data showed that the "DPI" (see footnote b of Table 5 for definition) for any of the 3 soils varied greatly with the different 12-hour periods. The variation in "Indices" during the eight 12-hour periods did not follow the same pattern in the 3 soils tested. Each soil produced an undulating pattern of variation, however, with one or more peaks always reached at the 1/8 dilution, thus producing a maximum of "DPI" of 8 that matched exactly with those of the 3 soils in Test II. For soil A, the "DPI" for the 8 periods, read 6 days after lemons were introduced to the cups, were respectively 2, 4, 4, 2, 8, 1, 1, and 1. For soil C, they were respectively 4, 8, 2, 8, 4, 1, 1, and 1. In soil A the peak of 8 was not reached until 2 days after water was added to the cups, whereas in soil C it was reached at 12 hours after water was added. The difference is thought to be due to the different moisture levels of the 2 soils at the start of the experiment, since soil C was saturated with water 2 days before the serial dilutions of the soils were made and the water apparently gave sporangium production in soil C a head start.

3) It was noted that with lemons used in the first five 12-hour periods, the "DPI" of all 3 soils obtained from reading at 6 days after water was added were identical to those from readings at 6 days after lemons were introduced. It seemed that lesion production must have been completed and was ready to be recorded at 4 days after lemons were in contact with the inoculum in the mud-water. The 6-day incubation was therefore believed to be more than adequate for a routine DP test.

4) The data further showed that infection on fruits placed in cups 60 or 72 hours after water was added was low, indicating that most of the sporangia and zoospores were produced during the first 2.5–3.0 days. After the end of the experiment, many of the dilution cups containing infective levels of inoculum were kept 18 more days, and fresh lemons were placed in them periodically. No brown rot lesions were found on any lemons introduced into the mud-water 8 days or more after water was added.

**Discussion.**—As in the Dimitman experiments (7), the lesions on the test fruits in the herein reported DP test were believed to be the result of infections by zoospores produced during the test. While it is conceivable that, theoretically, each viable fungus propagule in the soil would eventually bring about lesion development on a suitable host if a series of optimal

conditions were provided and sufficient time was allowed, not all propagules could produce sporangia and subsequent zoospores to induce brown rot lesions on the lemon fruit if the test was performed under one given set of environmental conditions and terminated after a given period. Therefore, the absence of brown rot on the fruits in a dilution cup does not necessarily indicate an absence of the fungus in the soil of that cup. Also, as in any isolation method where negative recovery does not necessarily mean the absence of the organism, an Index of zero for a soil does not always indicate an absence of the fungus in the soil sample tested. It may be conceived, however, that the amount of any fungus must be extremely minute.

Since environmental factors would affect zoospore formation of the fungus in the mud-water of the dilution cups, and thus the DPI levels, the disease potential of the fungus in one soil could not be compared with that of another soil tested at a different time unless the incubation conditions were the same. The accuracy of the test therefore depends largely on the precise control of test conditions, especially incubation temperature. In addition, success requires proper and careful choice of the test lemons. They should be mature enough and free of injury, for no soil fungi other than *Phytophthora* spp. are known to infect an uninjured lemon. Test fruits should be picked from branches high enough to avoid field contamination of *Phytophthora* spp. from soil in the orchard. Surface sterilization of the fruit, however, is not necessary. Preferably, test fruits should be those from trees that had not been sprayed with any pesticides since fruit set. When sources of the fruits are not known, the test lemons should be washed in 0.1*N* $HNO_3$ solution to remove copper fungicide residue possibly present on the surface. Fruits that have been processed in the packing house or waxed fruits from the market are unsatisfactory because of surface injuries. *Penicillium* and *Oospora* infections are common on such fruits.

The selective susceptibility of uninjured mature lemon fruits to *Phytophthora* infection, the characteristic lesions of brown rot and its odor on the fruits, and the briefness of the test make the lemon-trap technique an excellent method for detecting citrus Phytophthoras in the soil. Other materials tried, such as seeds, detached leaves, cut stem pieces of citrus, and hemp seeds, worked occasionally, but none possess all of the 3 desired features a lemon fruit does. Citrus fruits other than the bulky lemon might also perform satisfactorily, but unavailability might prohibit their routine use. In southern California lemon is grown in all regions and is available the year around, making it an excellent material for these tests.

In view of the data reported herein, the serial dilution end-point method appears satisfactory for estimating disease potentials of citrus Phytophthoras in the soil. The time required for completing all the test procedures for each soil sample is less than 30 minutes, and data recording is simple and quick. Tests not reported in detail were performed to compare the efficiency and accuracy of the split-cup as both the soil divider and the soil mixer with other more conventional dividing and mixing equipment (respectively torsion balance and mechanical rotating mixer). The results showed the split-cup far superior. It saved much time and effort without sacrificing accuracy. The DP test also proved sufficiently reproducible. It is admittedly only semiquantitative. Furthermore, the twofold differences in serial dilutions render it incapable of detecting the difference between 2 soils that vary only slightly in the level of disease potential of the fungus. Still, it has provided means for studying various ecological factors influencing the citrus Phytophthoras *in situ*, for comparing treatments in the labratory and greenhouse pot experiments (17, 20), and for determining the distribution of these fungi in small-scale field experiments (18). It is obvious that if an improper soil sample taken from a large area of uneven *Phytophthora* distribution was used in the test, the level of disease potential obtained from the test certainly would not indicate the correct degree of infestation of that area by these fungi. However, its doubtful capability in this connection should not be blamed on the serial dilution end-point method. Any method requires an accurate sampling plan.

With slight modifications, the serial dilution end-point method could be used for estimating the disease potentials of other *Phytophthora* spp. and certain other soil pathogens for which no suitable methods are now available. A preliminary experiment with bean seeds as traps, for determining levels of *Rhizoctonia solani* and *Pythium aphanidermatum* in the soil, showed that a modified method employing this same principle performed satisfactorily with these 2 fungi.—Department of Plant Pathology, University of California, Riverside.

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# VARIATION IN PERINEAL PATTERNS AND HOST SPECIFICITY OF MELOIDOGYNE INCOGNITA

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SUMMARY

Fourteen single egg-mass or larval isolates from 12 populations of *Meloidogyne incognita* from 9 states were propagated on tomato for 12 generations. Perineal patterns of many isolates ranged from that of typical *incognita*- to typical *acrita*-type. Characteristics specific for each isolate in the first generation persisted in the 12th generation. Different hosts (cotton, corn) did not influence morphology of the perineal pattern over a 20-month period. Progeny from individuals selected for a certain pattern type during 4 successive generations exhibited the general pattern variation of the original isolates. In general, very young females had patterns with finer striae than mature females. The *acrita*-type pattern, as shown in cross sections, was formed of regular cuticular striae and of additional foldings of the outer cuticle. Pattern was not correlated with ability of isolates to reproduce on cotton. Since morphological distinction between the subspecies is often uncertain and perineal pattern gives little information on the physiological behavior of a certain population, present division of the species into 2 subspecies seems to serve no practical purpose. It is therefore suggested that all populations exhibiting perineal patterns ranging from *incognita*- to *acrita*-type be considered one species, *M. incognita*. Most isolates reproduced slightly on resistant tobacco and tomato (Hawaii 5229). Three to 5 successive transfers on resistant plants resulted in clones of the isolates with increased reproductive rates on these plants. Clones built up on resistant tobacco reproduced on resistant tomato at the same rate as the original isolates. Attempts to cross several isolates gave no indication of amphigonic reproduction.

Following Chitwood's taxonomic revision of the genus *Meloidogyne* Goeldi, 1887 (2), several additional species were described and attention was focused on

**Table. 1.**—Sources, designation and characterization of the root-knot nematode populations and isolates.

| Population | Source | Isolate | Perineal pattern type[a] | | | Reproduced on cotton |
|---|---|---|---|---|---|---|
| | | | *acrita* | intermediate | *incognita* | |
| 1A | tomato, N. C. | 1A-1 | + | + | ++ | yes |
| 1B | cotton, N. C. | 1B-3 | ++ | + | — | yes |
| 1C | cotton, N. C. | 1C-1 | ++ | + | + | yes |
| 2A | tomato, Ga. | 2A-1 | + | — | — | yes |
| | | 2A-3 | + | — | — | no |
| 3A | okra, tomato, La. | 3A-2 | + | + | ++ | no |
| 4A | soil sample, Del. | 4A-4 | + | ++ | + | no |
| 5A | soil sample, Calif. | 5A-2 | + | ++ | — | no |
| 6A | soil sample, Miss. | 6A-4 | ++ | + | — | yes |
| 7A | parsnip, Ky. | 7A-6 | + | ++ | + | no |
| 7B | tobacco, Ky. | 7B-6 | + | ++ | + | no |
| 8A | soil sample, Ariz. | 8A-2 | + | ++ | + | yes |
| 9A | soybean, S. C. | 9A-1 | + | + | ++ | no |
| | | 9A-6 | + | + | ++ | yes |

[a] + = individuals with this type of pattern were present; ++ = most of the individuals had pattern of this type; — = individuals with this type of pattern were not present.

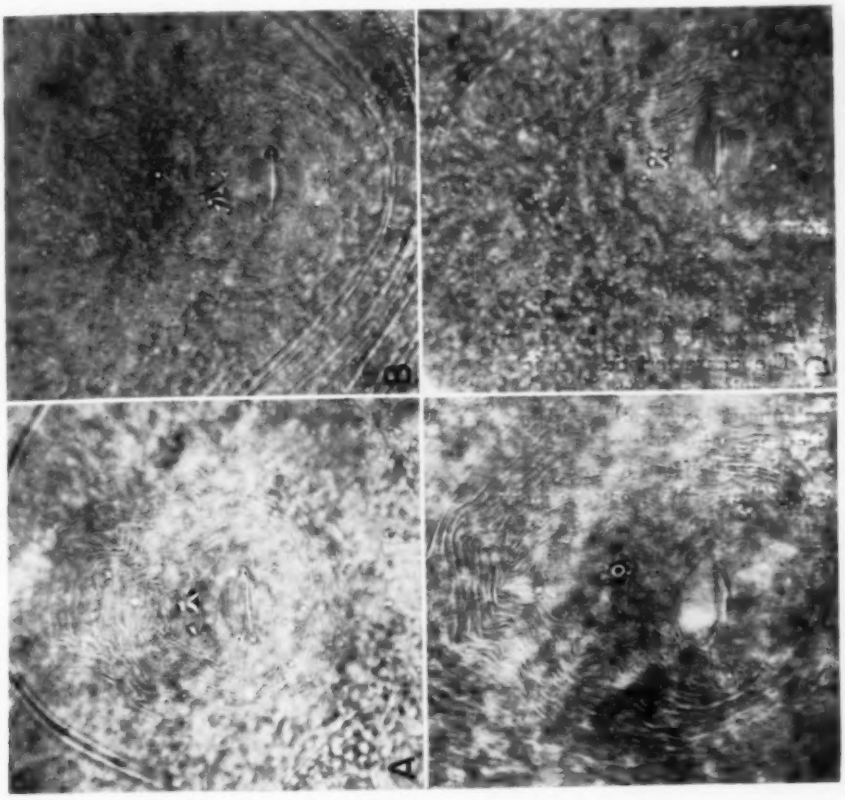

**Fig. 2.—A-D)** Variation in perineal patterns of very young females from the 12th generation of isolate 2A-3 of *M. incognita*. The lines forming the pattern are fine, zigzag, or wavy. (×650)

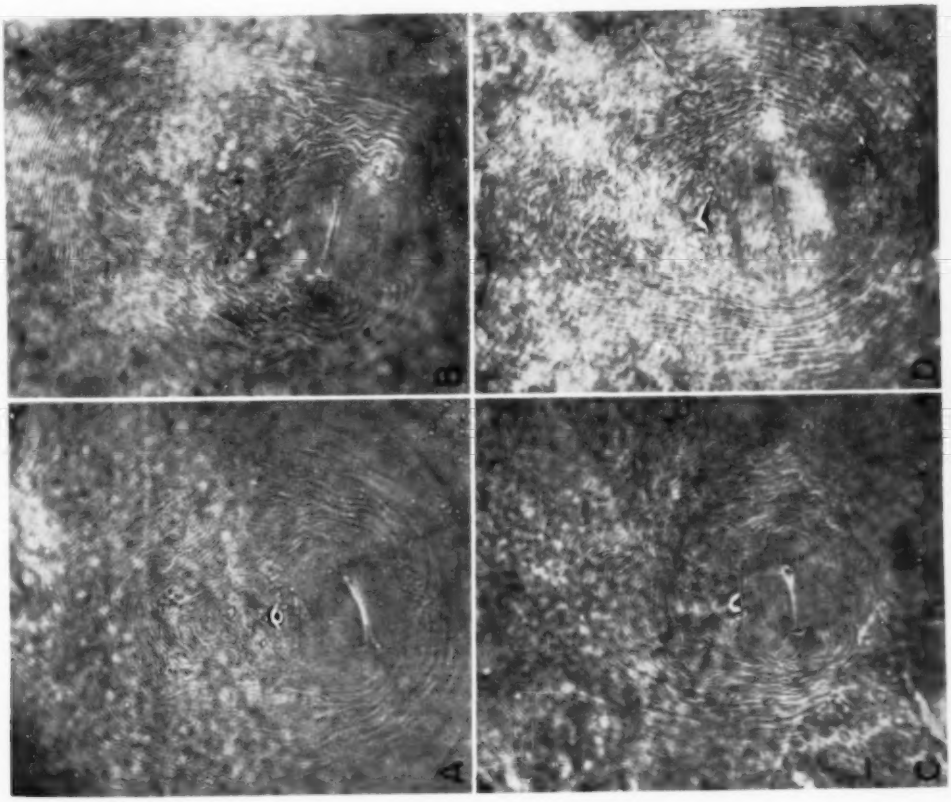

**Fig. 1.—A-D)** Variation in perineal patterns of very young females from the 12th generation of isolate 1A-1 of *M. incognita*. The lines forming the pattern are fine, zigzag, or wavy. (×650)

intraspecific variation. Among the several species, *Meloidogyne incognita* Chitwood, 1949, is most widely distributed by far, particularly in the temperate climates. Within this species Chitwood distinguished one variety, *acrita*, mainly on the basis of the morphology of the perineal pattern. Later, the variety was differentiated from the species on the basis of host specificity (10). According to the Rules of Zoological Nomenclature the variety has recently been considered as a subspecies and is now designated *M. incognita acrita*, in contrast to *M. incognita incognita* (3). Several investigations have indicated great variation in morphology and host specificity in this nematode species (6, 7, 8, 10). The characteristics originally considered specific for the subspecies are now proved to be variable and overlapping, leaving the taxonomic status of the subspecies uncertain.

Investigations were conducted of the extent of variation in the morphology of the perineal pattern and in host specificity of several isolates of *M. incognita*. Special emphasis was placed on a critical comparison of *M. incognita acrita* with *M. incognita incognita* to determine whether maintenance of the subspecies as distinct taxonomic entities is justified. Also studied was the source of variation and the mechanism of inheritance of certain characteristics of the isolates. A preliminary report has been published (15). Post-infection development and the mechanism of sex determination have also been investigated for these isolates, and are discussed in separate publications (13, 14).

**Literature review.**—The variability of the perineal pattern of the genus *Meloidogyne*, first recognized by Chitwood (2), was investigated by Dropkin (4) in larval isolates as well as in wild populations of *M. arenaria*, *M. incognita incognita*, and *M. incognita acrita*. He concluded that the general shape, and perhaps some of the details, of the perineal pattern are heritable characters. Sasser (10) found the morphology of the perineal pattern to be reliable for differentiating 4 *Meloidogyne* species. *M. incognita incognita* and *M. incognita acrita*, however, were similar, and difficult to differentiate by morphology of the perineal pattern alone. In distinguishing *M. incognita acrita* from *M. incognita incognita*, Taylor et al (12) placed emphasis on the shape and spacing of the striae of the arch of the perineal pattern: *M. incognita acrita* has an arch formed by widely spaced wavy striae; *M. incognita incognita* has striae that are closely spaced and wavy to zigzag.

Host specificity variation in *M. incognita incognita* and *M. incognita acrita* has been reported by a number of investigators. Differences in host plant reaction (degree or type of galling) in populations of this nematode species have been observed by Allen (1), Dropkin (5), and Martin (8). Further, considerable variation has been found in rate of reproduction and the ability of isolates to attack various cotton and soybean varieties (5, 7, 8). Populations of *M. incognita acrita* from 5 different sources varied in ability to attack *Sorghum almum* L. (7).

The existence of strains or races within the subspecies *acrita* has been reported by Sasser and Nusbaum (11). They found a population of *M. incognita acrita* that attacked cotton severely but did not attack tobacco, whereas a population in neighboring field plots attacked both. The existence of physiological races was recently demonstrated (6) on the basis of differential reaction of 8 collections of *M. incognita acrita* to 5 alfalfa varieties. Variation among individuals of a single egg-mass isolate was demonstrated in studies (9) with the resistant tomato variety Hawaii 5229. By successive transfers on resistant plants, clones of 2 isolates were obtained that reproduced readily on tomato plants resistant to the original isolates.

**Materials and methods.**—*General.*—Table 1 shows the sources (9 states) of soil samples or roots of host plants infected with *M. incognita* (used henceforth in this article to include both *M. incognita incognita* and *M. incognita acrita*). Stock cultures were established on young Rutgers tomato seedlings and maintained by transfer to new plants every 3 months. Six-inch pots filled with steam-sterilized soil were used in all experiments, and special care was taken to avoid contamination. The pots were kept in a greenhouse at about 29°C.

Three to six single egg mass and larval isolations were made from each population. After 45 days' growth, the seedlings were washed free of soil, and egg masses found in the roots were used to establish a second generation. The females were fixed in 2% formaldehyde for at least 3 days before dissection. The perineal region was mounted on slides in lactophenol (12). Inoculum was taken from the second generation to establish the third generation and to inoculate 3 seedlings each of the following plant species: Tobacco (var. DB-101), cotton (Coker 100W), and corn (Inbred T-8). After 50 days the plants were examined for galls and females with egg masses.

The perineal pattern of the first-generation females and the appearance of the root systems of the three test plants indicated the morphology and physiological (pathogenic) behavior of the populations and their isolates. Only one isolate from each population was maintained if there were no differences in type of perineal pattern or ability to attack the above-mentioned plant species. When variation was found, all isolates showing differences were propagated for further study. Table 1 shows the isolates maintained and their characteristics.

*Variation of the perineal pattern.*—Each isolate was propagated on tomato and cotton or corn for 10–12 generations by successive transfers every 42–45 days. Galled roots containing egg masses and larvae of the previous generation were used as inoculum. Perineal patterns of females with well-formed egg masses from the first and the 10th or 12th generations were studied in detail.

The following morphological characteristics were considered: a) height of arch; b) striae: straight (*acrita* type) or wavy, zigzag (*incognita* type); c) striae fine, evenly spaced (*incognita* type) or coarse

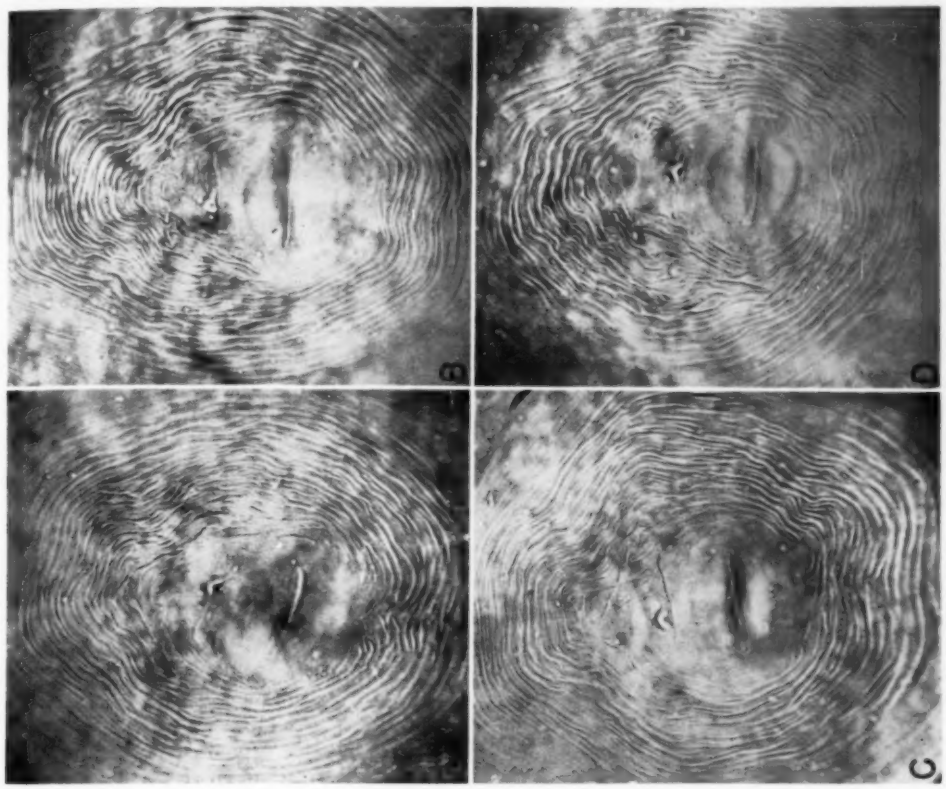


**Fig. 4.—A-D)** Variation in perineal patterns of egg-laying females from the first generation of isolate 2A-3 of *M. incognita*. (×650)

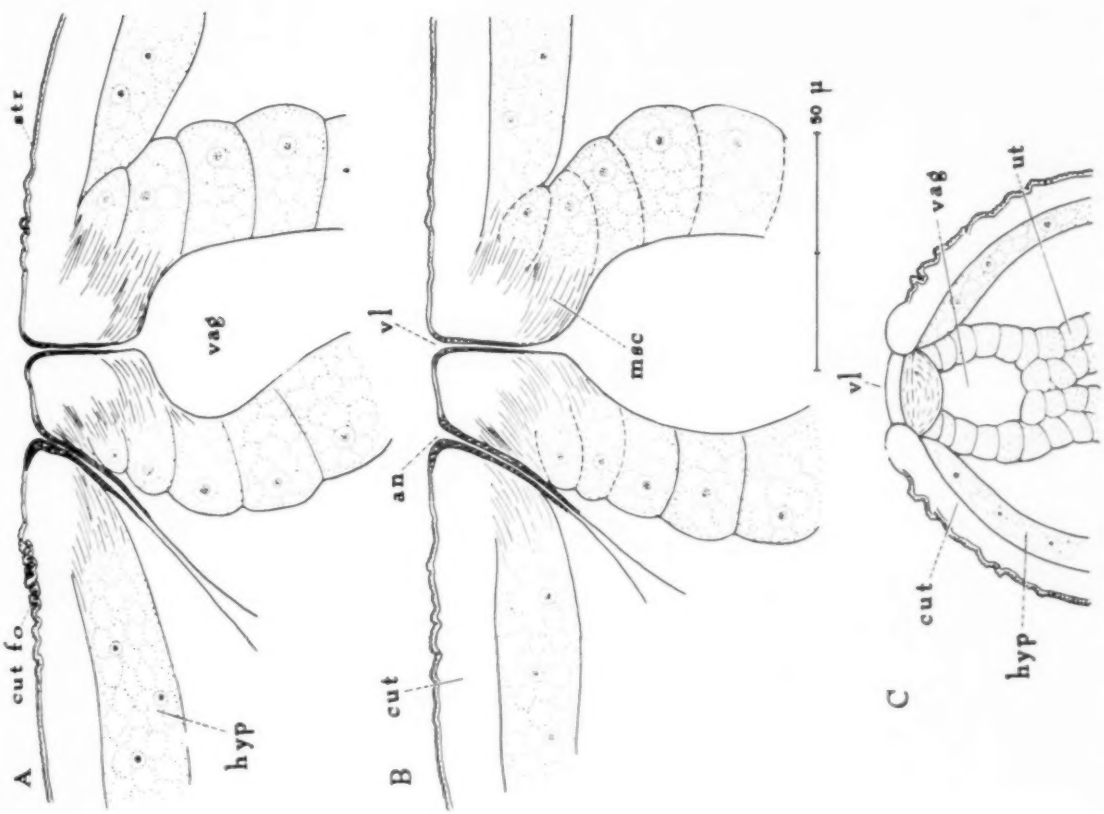


**Fig. 3.**—Cross sections through the perineal region of adult females of *M. incognita*. **A)** Egg-laying female of isolate 2A-3 with *acrita*-type pattern; **B)** Egg-laying female of isolate 1A-1 with *incognita*-type pattern; **C)** Young female of isolate 2A-1. an = anus; cut. = cuticle; cut.fo. = cuticular foldings; hyp. = hypodermis; msc. = muscle fibers; str. = striae; ut. = uterus, vag. = vagina; vl. = vulva.

**Table 2.**—Suitability of plants as hosts of several isolates of Meloidogyne incognita as indicated by infection classes.[a]

| Isolate | Type of perineal pattern | Corn | Cotton | Gherkin | | Bean | Peanut | Tomato | Tobacco | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Inbred T-8 | Coker 100 W | 82560 | West India | White Cornfield | NC2 | Hawaii 5229 | DB-101 | 8064 | 8080 |
| 1A-1 | incognita | 3–4 | 4 | 5 | 5 | 4–5 | 0 | 3 | 5 | 2 | 2 |
| 1B-3 | acrita | 4 | 4 | 4 | 5 | 4–5 | 0 | 3 | 5 | 1 | 1 |
| 1C-1 | acrita | 3–4 | 4 | 5 | 5 | 5 | 0 | 3 | 5 | 1 | 1 |
| 2A-1 | acrita | 4 | 4 | 5 | 4 | 5 | 0 | –[b] | 5 | 1 | 1 |
| 2A-3 | acrita | 3–4 | 1 | 5 | 5 | 4–5 | 0 | – | 5 | 1 | 1 |
| 3A-2 | incognita | 3–4 | 1 | 5 | 5 | 5 | 0 | – | 5 | 2 | 2 |
| 4A-2 | intermediate | 3–4 | 1 | 5 | 5 | 5 | 0 | 3 | 5 | – | – |
| 5A-2 | intermediate | 3 | 1 | 5 | 5 | 5 | 0 | 3 | 5 | 1 | 1 |
| 6A-4 | acrita | 3 | 5 | 5 | 5 | 5 | 0 | 3 | 5 | 1 | 1 |
| 7A-6 | intermediate | 3 | 1 | 4–5 | 4–5 | 5 | 0 | 3 | 5 | 1 | 1 |
| 7B-6 | intermediate | 4 | 1 | 4–5 | 4–5 | 4–5 | 0 | – | 5 | – | – |
| 8A-2 | intermediate | 3 | 4–5 | 5 | 5 | 5 | 0 | 3 | 5 | 1 | 2 |
| 9A-1 | incognita | 3 | 1 | 5 | 5 | 5 | 0 | 3 | 5 | 1 | 2 |
| 9A-6 | incognita | 3 | 4 | 5 | 5 | 5 | 0 | – | 5 | 1 | 1 |

[a] The following rating system was used: 0 = no evidence of infection; 1 = roots galled but larvae inside the galls dead; 2 = plants resistant—females with egg masses 1–10 per plant; 3 = slightly suitable host—females with egg masses 10–100 per plant, roots may be moderately galled; 4 = moderately suitable host—females with egg masses from 100 to moderately abundant, roots moderately to severely galled; 5 = highly suitable host—females with egg masses very abundant, roots moderately to severely galled.

[b] – = no test made.

(lines) in pairs (*acrita* type); d) limits of perineal pattern more or less well defined (*acrita* type) or outer striae progressively finer and merging into regular body striation (*incognita* type); and e) special characteristics such as anomalous striae or unusual pattern shape.

For each of the isolates 1C-1 and 3A-2, 15 to 20 small portions of tomato roots, each bearing one female with well-formed egg mass, were transferred individually to watch glasses containing a small amount of water. The egg masses were separated and the root portions containing female nematodes were transferred into vials filled with 2% formaldehyde. Twenty-four hours later the females were dissected and the perineal pattern examined. The one with the most typical *incognita*-type pattern and the one with the most typical *acrita*-type pattern were selected. The egg masses of these 2 individuals (mother individuals) were used to inoculate one tomato seedling each. Forty-five days later, 10–15 females with well-formed egg masses were isolated from each tomato plant, placed in watch glasses, and treated as described. The second generation was established with the egg mass of the female whose perineal pattern most resembled the pattern type of the mother individual. This selective isolation was completed through the 4th generation.

*Host suitability studies.*—Three-week-old seedlings were transplanted individually to 6-in. pots after galled tomato roots infected by single isolates of the nematode were added to the soil as inoculum. Each plant-isolate combination was replicated 5 times. After 50 days' growth, the roots were washed and examined macroscopically and under the dissecting microscope for galls and egg masses.

**Results.**—1) *Variation studies.*—A) *Development of the perineal pattern.*—The perineal region of young and mature females was examined in surface view and in cross sections made parallel or at right angles to the vulva. The perineal pattern became apparent in young females a few hours following the fourth molt, as the cuticle became distinct. The perineal pattern of very young females still enclosed in the larval cuticles of the previous stages differed markedly from that of mature, egg-laying females. The lines forming the pattern of young females were mostly fine, zigzag, or wavy, and the pattern usually resembled the *incognita*-type. Fig. 1 and 2 are photomicrographs of the perineal pattern of very young females of isolates 1A-1 and 2A-3, respectively dissected out of the same tomato roots as the mature females whose perineal patterns are shown in Figs. 8 and 5. The very young individuals differ in general pattern type from the mature females. This indicates that the morphology of the perineal pattern changes during development of the adult female.

In cross section the perineal pattern of mature females was found to be formed by regular cuticular striation as well as by foldings of the outer cuticular layer (Fig. 3-A). The coarse paired lines visible in surface view of *acrita*-type patterns obviously correspond to the grooves formed by two successive foldings. In *incognita*-type patterns, the foldings of the outer cuticular layer are fewer and less pronounced (Fig. 3-B). Most of the fine striae visible in surface view of *incognita*-type perineal patterns may correspond to the regular cuticular striation. A cross-section through the perineal region of a young non-egg-laying female is illustrated in Fig. 3-C. The cuticular foldings are forming at this stage.

B) *Stability of the perineal pattern.*—When the perineal patterns of 30–50 individuals of the 10–12th generations on tomato were compared with those of an approximately equal number of individuals of the first generation, some pattern characteristics were found to persist from the first to the last generation of each

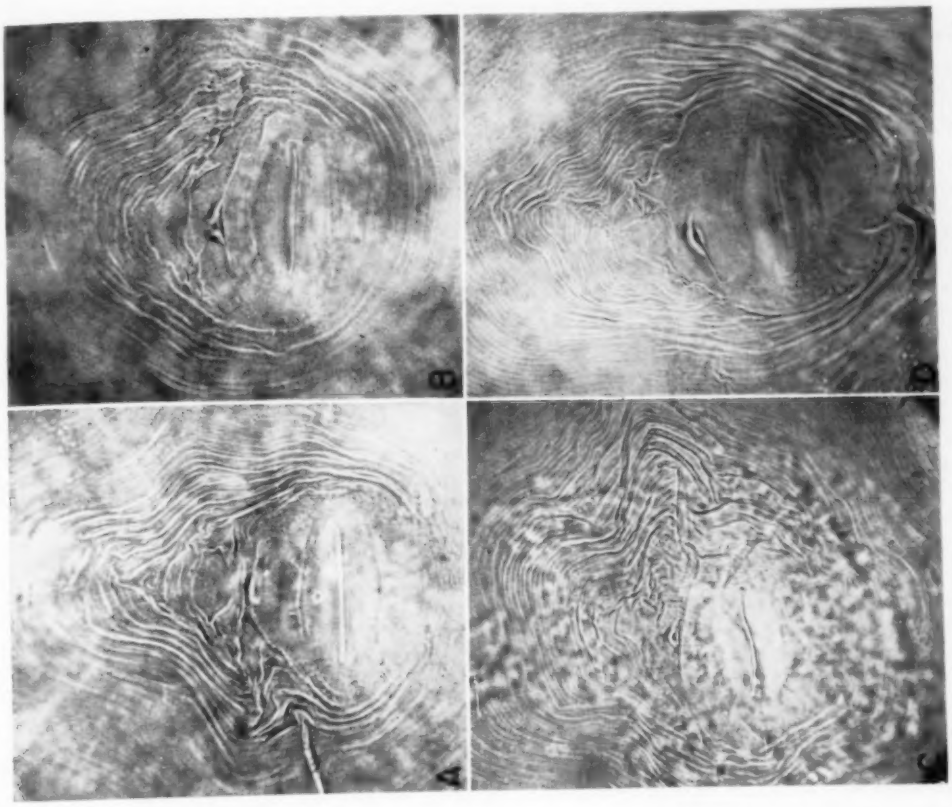


**Fig. 6.—A-D)** Variation in perineal patterns of egg-laying females from the first generation of isolate 2A-1 of *M. incognita*. (×650)

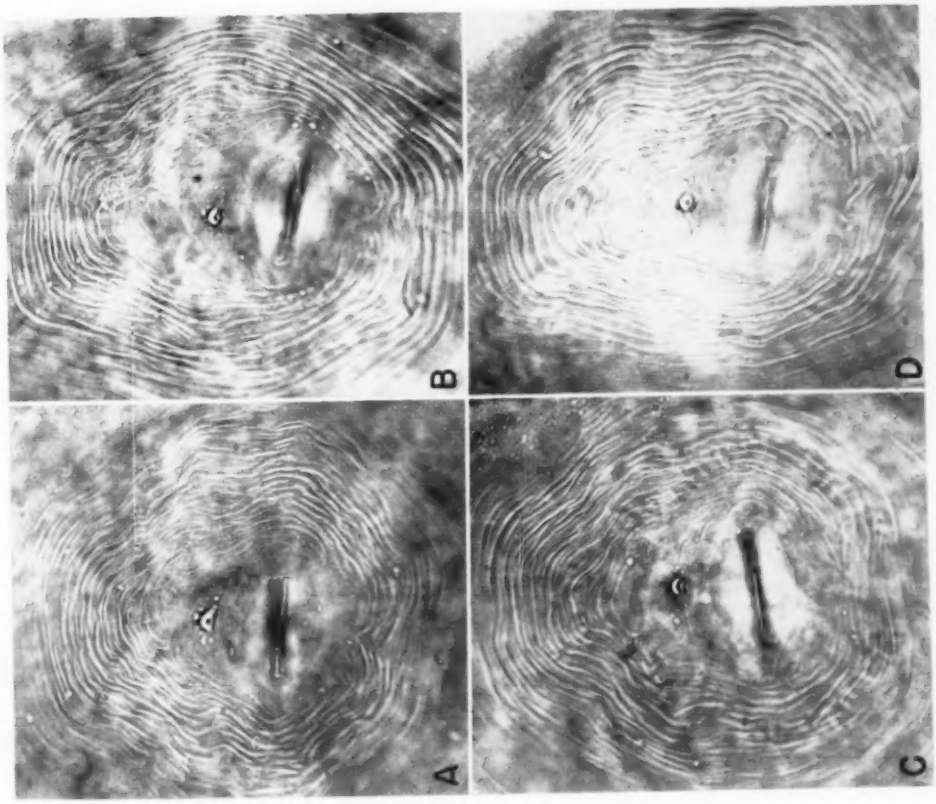


**Fig. 5.—A-D)** Variation in perineal patterns of egg-laying females from the 12th generation of isolate 2A-3 of *M. incognita*. (×650)

**Table 3.**—Root-knot infection classes of 3 tobacco varieties or lines inoculated with several isolates of *Meloidogyne incognita*.

| Isolate | Tobacco line or variety | | |
|---|---|---|---|
| | Susceptible DB-101 | Resistant Bel 6-28 | Resistant 8071 |
| 3A-2—Original | 5[b] | 2 | 2 |
| —Clone B[a] | 5 | 4 | 4 |
| 9A-1—Original | 5 | 2 | 2 |
| —Clone B | 5 | 4 | 4 |

[a] B represents the clone of the isolate that had been built up on resistant tobacco.
[b] For rating system see Table 2.

isolate. Two were selected to illustrate all 14 isolates:

a) *Isolate 2A-3.*—In the first generation this isolate showed the most typical *acrita*-type perineal patterns, with little variation. Uniformity of the patterns is illustrated in Fig. 4-A–D. In the 12th generation the same isolate showed a very similar type of perineal pattern, and variation was again very small (Fig. 5-A–D).

b) *Isolate 2A-1.*—In the first generation this isolate showed considerable variation. Only half of the perineal patterns were the typical *acrita*-type. Most of the others had irregular striae (Fig. 6-A,C,D); a few showed some of the characteristics of *M. hapla* (Fig. 6-B). The over-all variation was the same in the 12th generation. Typical *acrita*-type patterns made up about the same proportion, and the other two pattern types were also present. Patterns of the irregular type are illustrated in Fig. 7-A,B, and one showing some characteristics of *M. hapla* in Fig. 7-D.

C) *Effect of host plant on perineal pattern.*—When perineal patterns of individuals propagated 18–22 months on cotton or corn are compared with those of individuals of the same isolate propagated for an equal period on tomato, no substantial effect of host on pattern could be detected. Characteristics of the perineal pattern specific for each isolate in the first generation persisted through the 12th generation on tomato and cotton or corn. A single isolate is used to illustrate this:

a) *Isolate 1A-1.*—The perineal patterns of this isolate were largely of the *incognita*-type in the first as well as in the 12th generation on tomato (Fig. 8-A–C). There were, however, a few patterns of the *acrita*-type (Fig. 8-D) and several intermediates not characteristic of either type. The same isolate after 20 months on cotton showed similar variation and pattern types (Fig. 9).

D) *Attempt to obtain a family of individuals with uniform type of perineal pattern.*—The progenies of individuals selected for a certain type of perineal pattern showed in the 4th generation pattern characteristics similar to those of the original isolate.

a) *Isolate 3A-2.*—This isolate exhibited a perineal pattern of the *incognita*-type, though not typical; few patterns were of the *acrita*-type. The female with the *acrita*-type pattern illustrated in Fig. 10-A was selected as mother individual to initiate the experiment. For each subsequent generation the individual with the most typical *acrita*-type pattern was selected. Most individuals of the 4th generation had a perineal pattern typical of the original isolate. No shift toward *acrita*-type could be detected. Three patterns of the 4th generation are illustrated in Fig. 10-B–D.

Results were similar from selecting for the most typical *incognita*-type pattern in isolates 3A-2 and 1C-1 and *acrita*-type pattern in isolate 1C-1.

b) *Comparing 2 isolates of the same population.*—Two isolates (9A-1, 9A-6) selected from the stock culture 9A of South Carolina showed similar perineal pattern types. Isolate 9A-1 did not reproduce on cotton, whereas isolate 9A-6 did. Both isolates showed a variation of perineal pattern from typical *acrita* (Fig. 11-A, 12-D) to typical *incognita* (Fig. 11-D, 12-C) type. In addition they possessed a number (20–30%) of perineal patterns with a very low arch. Most of these patterns were almost circular (Fig. 11-C, 12-B,C) or were wider than high (Fig. 11-B). This unusual pattern type persisted in the 12th generation, showing a slight increase in percentage over the first generation.

2) *Host suitability studies.*—Ten plant species or varieties were selected for investigation of the physiological behavior (host specificity) of 14 isolates of the parasite. Variation in rating of replicate plants of each plant-parasite combination was usually small. All plants could be classified in the same infection class. In the few cases showing considerable variation, the experiment was repeated. The results are summarized in Table 2. Half of the isolates reproduced on cotton to varying degrees. Although 2A-1 and 2A-3 were isolates of the same population, they differed distinctly in ability to reproduce on cotton. The same was true for isolates 9A-1 and 9A-6. Four of the 5 isolates with *acrita*-type pattern and 2 of the 4 with *incognita*-type pattern reproduced on cotton. Corn was a slightly or moderately favorable host for all isolates. All isolates reproduced slightly on resistant tomato. Two isolates with *incognita*-type pattern (1A-1 and 3A-2) reproduced slightly on both breeding lines of resistant tobacco (root-knot-resistant breeding lines developed at the Tobacco Research Station, Oxford, N. C.). Isolates 9A-1, with *incognita*-type pattern, and 8A-2, with

**Table 4.**—Root-knot infection classes of 2 tomato varieties inoculated with several isolates of *Meloidogyne incognita*

| Isolates | Tomato variety | |
|---|---|---|
| | Hawaii 5229 | Rutgers |
| 1A-1—Original | 3[b] | 5 |
| —Clone B[a] | 3–4 | 5 |
| 1B-3—Original | 3 | 5 |
| —Clone B | 3–4 | 5 |
| 7A-6—Original | 2 | 5 |
| —Clone B | 4 | 5 |
| 9-A1—Original | 3 | 5 |
| —Clone B | 4–5 | 5 |

[a] B represents the clone of the isolate that had been built up on resistant tomato.
[b] For rating system see Table 2.

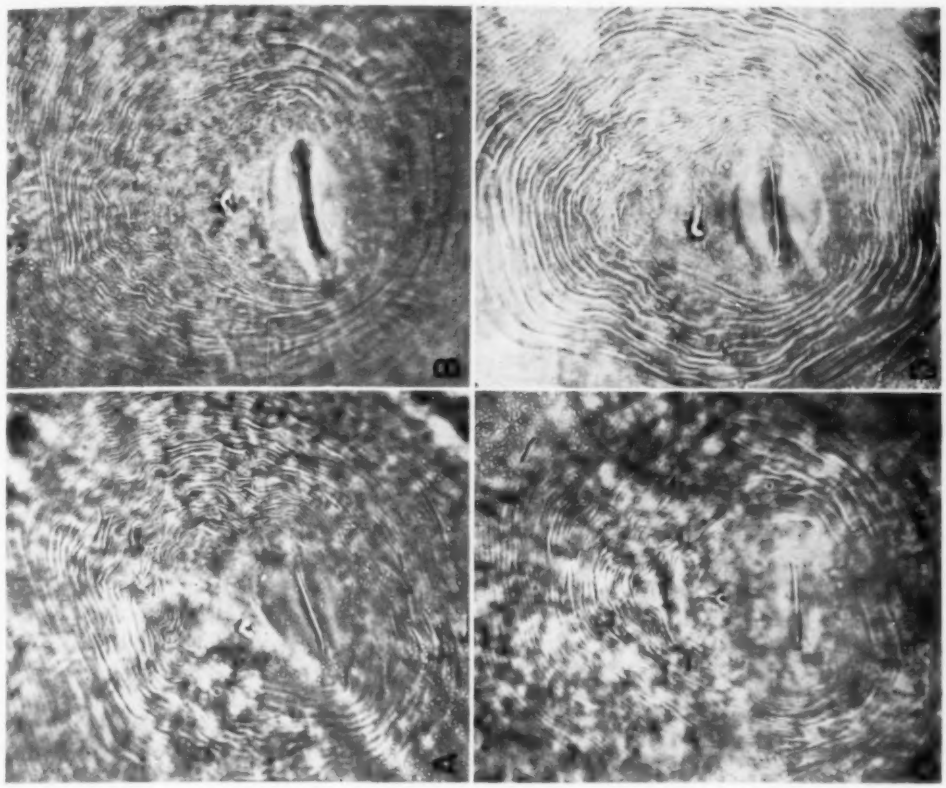


**Fig. 8.—A-D)** Variation in perineal patterns of egg-laying females from the 12th generation of isolate 1A-1 of *M. incognita*. (×650)

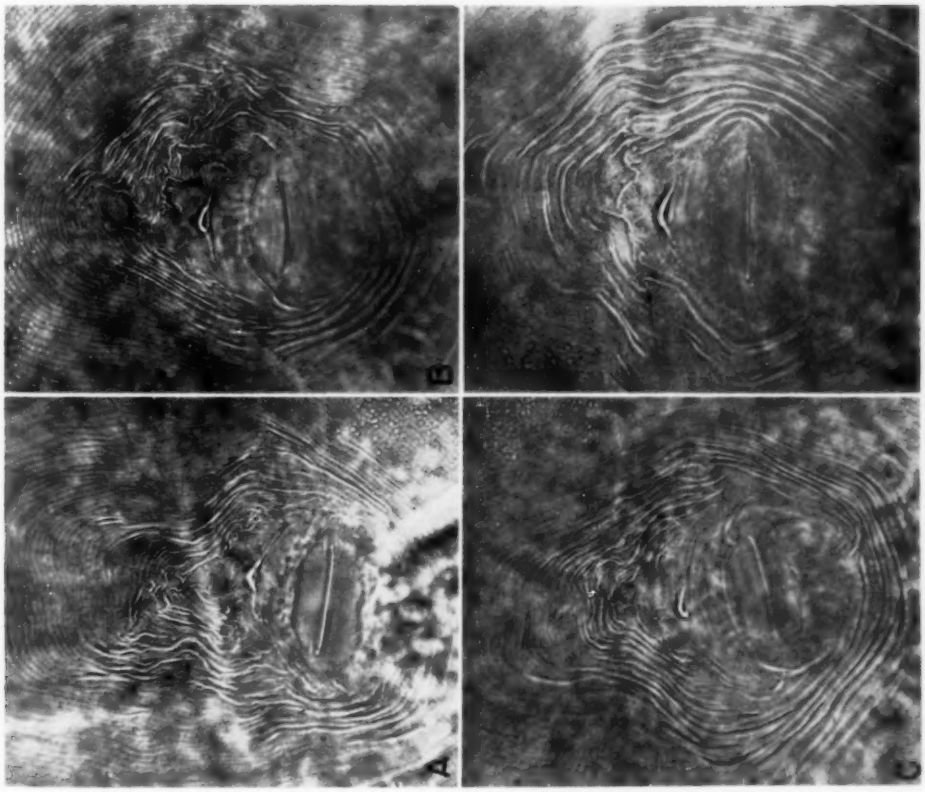


**Fig. 7.—A-D)** Variation in perineal patterns of egg-laying females from the 12th generation of isolate 2A-1 of *M. incognita*. (×650)

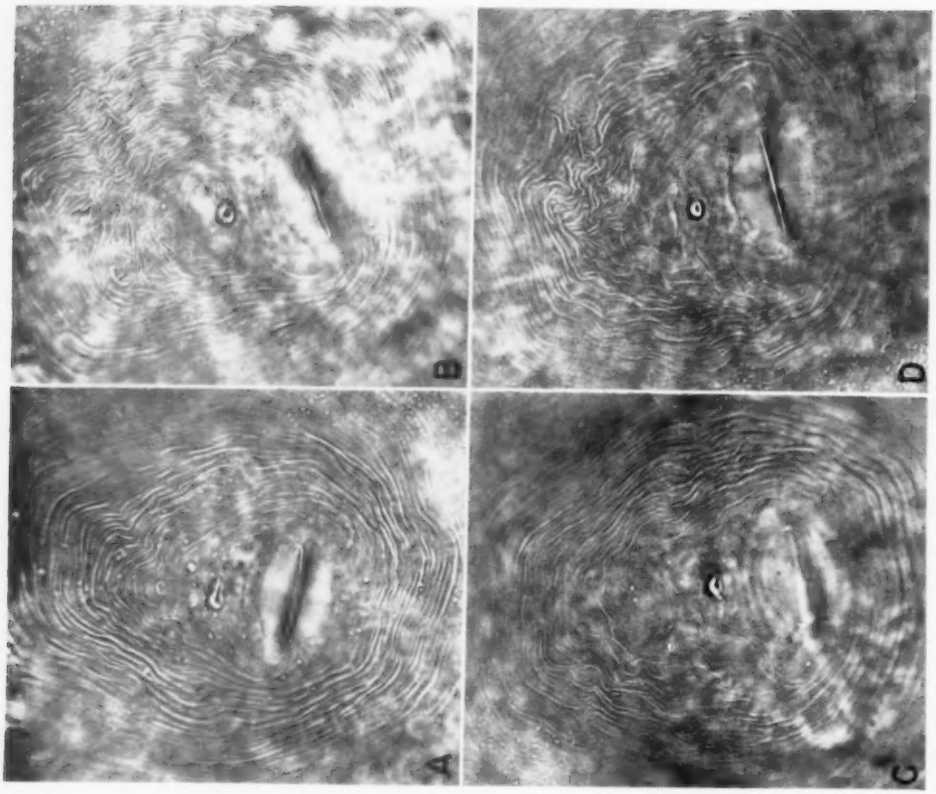

**Fig. 10.**—Perineal patterns of egg-laying females of isolate 3A-2 of *M. incognita*. **A)** Mother individual; **B-D)** Individuals from the 4th generation. In each generation the individual with the most typical *acrita*-type pattern was used for establishing the next generation. (×650)

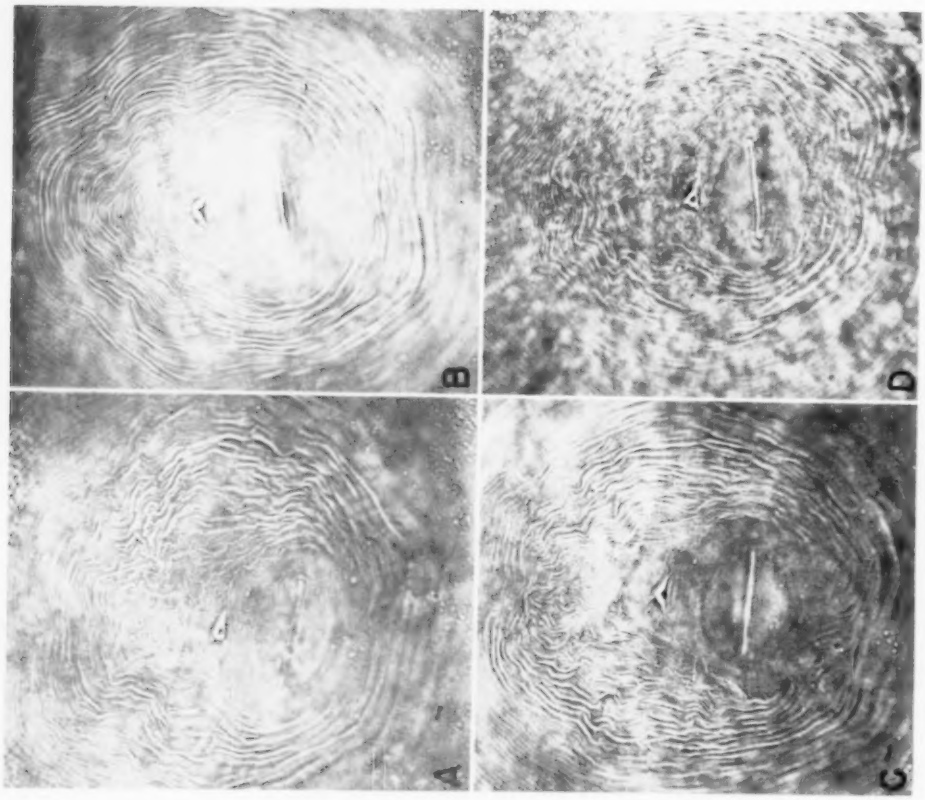

**Fig. 9.**—**A-D)** Variation in perineal patterns of egg-laying females of isolate 1A-1 of *M. incognita* propagated for 20 months on cotton. (×650)

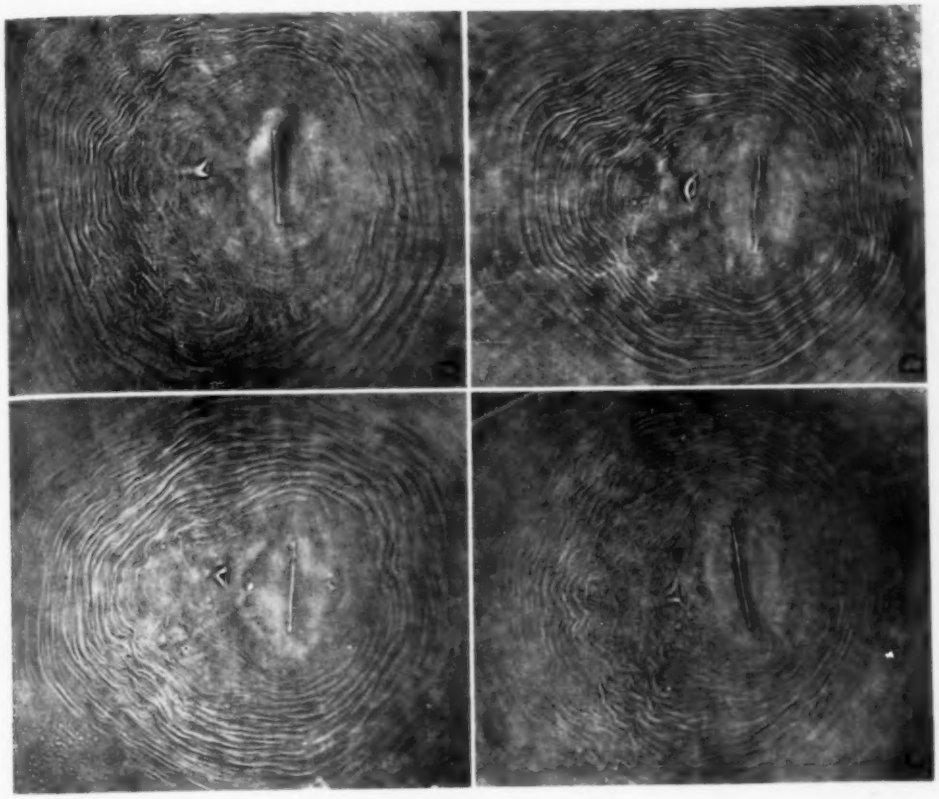

**Fig. 12.—A-D)** Variation in perineal patterns of egg-laying females from the first generation of isolate 9A-6 of *M. incognita*. (×650)

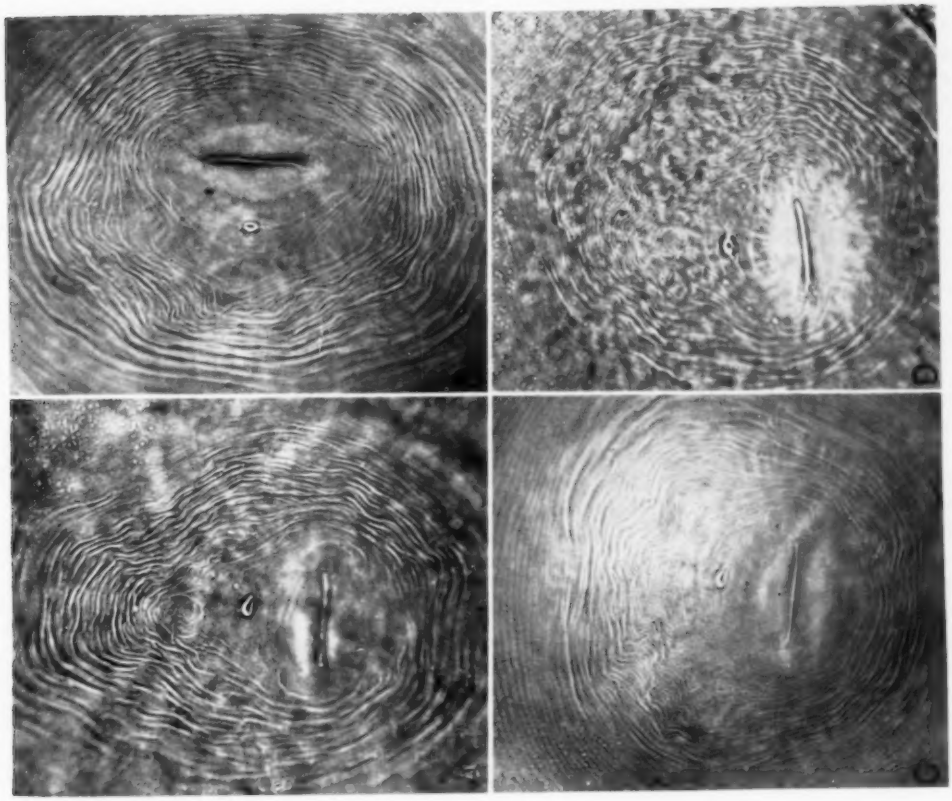

**Fig. 11.—A-D)** Variation in perineal patterns of egg-laying females from the first generation of isolate 9A-1 of *M. incognita*. (×650)

intermediate-type pattern, reproduced slightly on only one breeding line. All isolates with *acrita*-type pattern failed to reproduce on either breeding line.

3) *Studies on resistant tobacco.*—In the host suitability studies, some isolates of the parasite reproduced slightly on resistant tobacco (Table 2). Inoculum from resistant plants was increased on susceptible tobacco (DB-101) and then used again to inoculate resistant plants. In this second transfer two isolates gave rise to significantly more egg masses (root-knot infection class = 3) than in the first inoculation on resistant plants (infection class = 2). Individuals of each isolate thus obtained from resistant tobacco were designated B clones, in contrast to the original isolates, propagated continuously on susceptible tobacco.

The B clones as well as the original isolates were used for a third time to inoculate resistant and susceptible tobacco. Results are summarized in Table 3. In the third transfer the B clones were able to reproduce on resistant tobacco more readily than the original isolates. Resistant tobacco lines were, in fact, moderately susceptible hosts for B clones. They still possessed, however, a small degree of resistance in comparison with the susceptible variety DB-101.

The above B clones were later inoculated to the resistant tomato variety Hawaii 5229 and to the susceptible tomato variety Rutgers. After 2½ months the root systems of the plants were examined. The root-knot infection class was 3 for resistant plants and 5 for susceptible plants. Thus, the B clones of the isolates able to overcome resistance on tobacco behaved on resistant tomato like the original isolates.

4) *Studies on resistant tomato.*—The resistant tomato line Hawaii 5229 was attacked slightly by all isolates of *M. incognita* tested (Table 2). Some of the isolates were propagated for a second generation on resistant tomato but showed no increase over the original isolates in reproductive rate. Four isolates, however, were propagated for three or four generations on resistant tomato (they are designated clones B) and then compared with the original isolates. The results are summarized in Table 4. The B clones of isolates 7A-6 and 9A-1 increased rate of reproduction over that of the original isolates. A slight increase was observed in clones B of 1A-1 and 1B-3.

5) *Source of variation and the mechanism of inheritance of various characteristics.*—In breeding experiments, males of different isolates of *M. incognita* and males of *M. hapla* and *M. javanica* were added to the soil where tomato plants were growing with very young females of *M. incognita* in their roots. The males were later found associated with the females. Males were absent in the controls. In some cases sperm was found in the spermathecae of the females. The sperm entered the oocyte during the prophase stage and remained at one pole of the egg until the first telophase. Its further role was not detected. The morphological and physiological characteristics of the offspring of the attempted crosses gave no indication of amphigonic reproduction.

**Discussion.**—The perineal pattern has been considered to be formed by simple cuticular striae (2, 3, 12). Cross sections through the perineal region of mature females, however, reveal that the perineal pattern consists of both the regular cuticular striae and additional foldings of the outer cuticular layer. This structure may suggest an explanation concerning the formation of the perineal pattern. In very young females the perineal region is marked by regular cuticular striae and is hemispherical. As the female widens in the middle of the body, the perineal region changes from hemispheric to plain. The change may cause expansion of the inner cuticular layers and a folding of the outer cuticular layer. The perineal pattern thus changes with maturation of the individuals, and taxonomic examinations of perineal patterns should be limited to females of a certain age (e.g., egg-laying females).

Of the isolates studied, in the first generation some showed *acrita*-type patterns and others *incognita*-type, but most possessed intermediate patterns characteristic of neither type or else patterns ranging from *acrita*- to *incognita*-type. On that basis, then, *M. incognita incognita* could not always be distinguished from *M. incognita acrita*.

Most of the isolates showed several specific pattern characteristics by which they could be morphologically distinguished from the other isolates. These specific characteristics persisted from the first to the 10th or 12th generation of the isolates propagated on 3 different hosts. This suggests that at least some of the pattern characteristics may be genetically controlled. Propkin (4) reached a similar conclusion. The great variation, however, of the perineal pattern in the offspring of a single female, which in the absence of males reproduces parthenogenetically (ameiotic type; unpublished data of the senior author), cannot be explained on a genetic basis. It is possible that the great variation in pattern characteristics may result from rate of development of the nematode, its position in the host tissue, and other factors.

Patterns of most isolates were distributed within the limits of the *incognita-acrita* type. A few isolates, however, showed a greater variation. One had a high percentage of perineal patterns with very low arch, atypical of either *incognita*- or *acrita*-types. Another isolate possessed perineal patterns of *acrita*-type with many irregular striae, and also patterns with some of the characteristics of *M. hapla*. In other respects, however, the isolate resembled *M. incognita*. It attacked corn but not peanuts, and possessed the same chromosome number and other cytological characteristics of *M. incognita*.

All isolates exhibiting *acrita*-type perineal patterns failed to reproduce on resistant tobacco, whereas some of the isolates with *incognita* or intermediate-type patterns reproduced slightly. The differences between isolates may be partly due to insufficient testing. With more plants some egg masses might have been produced on resistant tobacco by the other isolates too.

No correlation was found between perineal pattern

and ability to reproduce on cotton. Other workers (7, 8, 11) reported similar results. In addition, van der Linde (7) found root-knot nematode populations with *incognita*-type pattern parasitizing *Lycopersicon peruvianum* L., although, according to Sasser's "host reaction test" (10), they should not reproduce on that species.

Since morphological distinction between the subspecies is often uncertain and perineal pattern gives little information on the physiological behavior of a certain population, present division of the species into 2 subspecies seems to serve no practical purpose. It is therefore suggested that all populations exhibiting perineal patterns ranging from *incognita*- to *acrita*-type be considered one species, *M. incognita*. Further investigations may offer additional information by which stable morphological characteristics can be correlated to physiological behavior. Such correlation may be found easily in populations of a certain geographical area, but is likely to be of little value for populations from diverse areas. Study of local populations may prove helpful to understanding root-knot nematode problems in the local area. In living organisms with a limited potential for migration and predominantly parthenogenetic mode of reproduction, the local population may be the most important and useful taxonomic unit.

Successive transfers of several isolates on resistant tomato or tobacco yielded clones that showed increased reproductive rates on resistant plants. This suggests a physiological variation in the respective original isolates. Riggs and Winstead (9) reported similar results. The mechanism by which clones of the isolates developed under the selection pressure of a resistant host variety is difficult to understand since ameiotic parthenogenesis was the main mode of reproduction, at least under the conditions of these experiments.

Attempted crosses between isolates of *M. incognita* failed, though that does not prove that amphigonic reproduction can never occur. Further investigations will be necessary before mode of reproduction in this species is clearly understood.

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# GENETICS OF THE ALLELIC SERIES AT THE $Ml_a$ LOCUS IN BARLEY AND CULTURES OF ERYSIPHE GRAMINIS F. SP. HORDEI THAT DIFFERENTIATE THESE ALLELES

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SUMMARY.

Information on the inheritance of the reactions of barley varieties Algerian (C.I. 1179) and Ricardo (C.I. 6306) to infection with cultures CR3 and 061 of *Erysiphe graminis* f. sp. *hordei* and the inheritance of pathogenicity of those 2 cultures on those 2 varieties was used to determine the genotypes of the hosts and pathogens. Gene $Ml_a$ in Algerian conditioned resistance to both cultures of the pathogen. Since the gene in Ricardo that conditioned resistance to culture CR3 but not culture 061 was either allelic or less than 1% crossover unit from $Ml_a$, it was considered to be allelic and designated $Ml_a3$. Culture CR3 had 2 avirulent genes, $A_a$ and $A_a3$, complementary to resistant genes $Ml_a$ and $Ml_a3$ in the host. Culture 061 had avirulent gene $A_a$ complementary to resistant gene $Ml_a$ and virulent gene $V_a3$ complementary to resistant gene $Ml_a3$ in the host.

**Introduction.**—The gene has gradually replaced the variety as the unit-source of resistance in developing disease-resistant varieties. Information on race-gene relationship has made it possible in many cases to provide a wide race coverage by combining specific genes. Mounting evidence of differential race coverage by various alleles of a given gene or locus, however, indicates that the gene should be replaced by the allele as a unit of resistance. This refinement has been made possible by an accumulation of information on the genetics of host resistance through the utilization of selected races or cultures of the pathogen.

Information accumulated on the genetics of resistance in the host is considerable, but little is known about the genetics of virulence within the pathogen. New races could be predicted more easily if we had a clearer picture of the variation existing within the pathogens, as well as the genetic basis of this variation. The plant breeder could then develop a positive approach in developing varieties that are resistant not only to existing but to pathogenic strains of the pathogen that might occur in the future.

This study differentiated between 2 alleles conditioning resistance to *Erysiphe graminis* DC. f. sp. *hordei* Em. Marchal in 2 varieties of barley by utilizing cultures of the pathogen with differential pathogenicity. The segregation and interrelationship of the genes for reaction in the host and the genes for pathogenicity in the pathogen were determined, and the significance of the relationship is discussed.

**Review of literature.**—Schaller and Briggs (11) and Moseman and Schaller (8) recently presented comprehensive reviews on the genetics of reaction of barley to powdery mildew. Only the information pertinent to the present study is discussed herein. In 1932 Mains and Martini (4) reported the variety Algerian (C.I. 1179; accession number of the Cereal Crops Research Branch, Crops Research Division) to be resistant to North American races 1, 2, and 3 of powdery mildew. Briggs and Stanford (1) found that the resistance in Algerian to race 3 in California was conditioned by one gene, which they designated $Ml_a$. This gene was inherited independently of the genes that condition resistance to race 3 in the following varieties: $Ml_h$ in Hanna (C.I. 906), $Ml_g$ in Goldfoil (C.I. 928), $Ml_p$ and $Ml_y$ in Chinerme (C.I. 1079). It was found, however, to be linked with the $Ml_k$ gene in Kwan (C.I. 1016), with a crossover value of 9.8± 4.78%. In 1955 Schaller and Briggs (11) showed that the $Ml_p$ gene in the varieties Chinerme and Psaknon (C.I. 6305) was loosely linked with $Ml_a$, with a crossover value of 41.5±2.7%. They also reported that the gene in the variety Black Russian (C.I. 2202) was allelic to the $Ml_a$ gene in Algerian, and designated it $Ml_a2$. Hiura et al (2) reported an allelic series of genes in the varieties Algerian and Black Russian, which they designated respectively $Ml_9$ and $Ml_{10}$. They also found that the genes $Ml_1$ in *H. s. nigrum* and $Ml_5$ in the variety Vaughn were either allelic or closely linked with the $Ml_9$ allele in Algerian.

The variety Ricardo (C.I. 6306) was reported by Newton and Cherewick (10) to be resistant to several races of the pathogen. In preliminary studies, the junior author found (unpublished data) that the resistance in Ricardo to race 3 was conditioned by a single gene, which appeared to be the same as gene $Ml_a$ in Algerian except for the degree of resistance it imparted. This difference, though apparent in the varieties, was more striking in their derivatives, especially with respect to the level of dominance in the heterozygous condition. In 1958, Moseman and Schaller (7) showed that the gene in Ricardo was an allele of the $Ml_a$ gene, and designated it $Ml_a3$. They also found (8) that the gene $Ml_a3$ in Ricardo conditioning resistance to culture CR3 of race 3 conditions resistance to cultures A8 of race 6, 28A1 of race 8, A9 of race 9, R11 of race 11, and CAN12 of race 12. Moseman and Starling (9) reported that the same gene in Ricardo conditioned resistance to cultures BC3 of race 3, T4 of race 4, NC9 of race 9, and Vall of race 11.

The interrelationship between the genes conditioning reaction in barley and the genes conditioning pathogenicity in powdery mildew has not been studied extensively. Moseman (6) has shown that for the 2 genes $Ml_g$ and $Ml_k$, respectively conditioning reaction

**Table 1.**—Seedling reaction of $F_2$ plants and $F_3$ families from crosses of 8 barley varieties with Ricardo to infection with culture CR3 of *Erysiphe graminis hordei*.

| Generation | Parent or hybrid | Seedling reaction (number) | | | Expected ratio | Value of P for ratio indicated |
|---|---|---|---|---|---|---|
| | | Resistant | Heterozygous or segregating | Susceptible | | |
| | Ricardo | 360 | | | | |
| $F_2$ | Ricardo × Manchuria | 66 | 159 | 70 | 1:2:1 | >0.30 |
| $F_3$ | Ricardo × Manchuria | 9 | 25 | 16 | 1:2:1 | >0.30 |
| $F_2$ | Ricardo × Atlas | 486 | | 165 | 3:1 | >0.50 |
| $F_3$ | Ricardo × Atlas | 66 | 110 | 44 | 1:2:1 | >0.10 |
| $F_2$ | Ricardo × Goldfoil | 385 | | 33 | 15:1 | >0.10 |
| $F_2$ | Ricardo × Hanna | 332 | | 16 | 15:1 | >0.10 |
| $F_2$ | Ricardo × Chinerme | 364 | | 2 | 63:1 | >0.10 |
| $F_2$ | Ricardo × Psaknon | 371 | | 15 | 15:1 | >0.05 |
| $F_2$ | Ricardo × Kwan | 579 | | 0 | 15:1 | <0.01 |
| $F_3$ | Ricardo × Kwan | 58 | 8 | 0 | 7:8:1 | <0.01 |
| $F_2$ | Ricardo × Algerian | 366 | | 0 | 15:1 | <0.01 |
| $F_3$ | Ricardo × Algerian | 300 | 0 | 0 | 1:2:1 | <0.01 |

in the barley varieties Goldfoil and Kwan, there are 2 complementary genes, $V_g$ and $V_k$, for pathogenicity in the pathogen.

**Materials and methods.**—Nine varieties of barley were used in this study. Eight of them—Atlas (C.I. 4118), Algerian, Ricardo, Goldfoil, Hanna, Kwan, Chinerme, and Psaknon—came from the source used in previous studies with California race 3 of powdery mildew. The other variety, Manchuria (C.I. 2330), obtained from G. A. Wiebe, was susceptible to both cultures CR3 and 061 of the pathogen. The variety Atlas was susceptible to culture CR3 but resistant to culture 061.

Ricardo was crossed with Atlas and Manchuria (susceptible to culture CR3) and the other 6 varieties (testers for 6 of the genes conditioning reaction to culture CR3 of powdery mildew). Also crossed with Manchuria was Algerian. Segregation of the seedling reaction of $F_2$ and $F_3$ progenies from these crosses to infection with cultures CR3 and 061 was studied.

Two cultures of powdery mildew, CR3 and 061, were used in this study. Culture CR3 originated as a subculture of the original culture of California race 3 and, therefore, should be comparable in pathogenicity to that culture. Culture 061 was obtained from a single ascospore from a cleistothecium on the leaf of a plant grown in 1956 at Ithaca, New York.

The pathogenicity of these 2 cultures and of the progeny from a cross between them was studied. The technique of crossing cultures of powdery mildew and obtaining new cultures from cleistothecia was described by Moseman (6).

All tests were made on plants growing either in greenhouse benches at about 70°F or in a growing room at 66–69°F with 550 ft-c of light from cool, white, fluorescent tubes for 16 hours a day. The methods of inoculation and of recording the reaction of plants to infection were similar to those published in 1956 (5). The designations of the genes conditioning reaction in the host and conditioning pathogenicity in the pathogen are as suggested by Moseman (6).

**Results.**—*Genetics of reaction in the host.*—Table 1 shows the segregation for reaction to infection with culture CR3 of seedlings from the $F_2$ and $F_3$ generations of crosses of Ricardo with Manchuria and Atlas, both susceptible to culture CR3, and with the 6 varieties used as testers for genes conditioning the reaction to culture CR3.

The ratio of resistant to susceptible plants to infection with culture CR3 in the $F_2$ population from the crosses of Ricardo with the susceptible varieties Manchuria and Atlas gave a good fit to the 3:1 or 1:2:1 ratios expected for monofactorial segregation. Tests with the $F_3$ families confirmed the single gene-segregation indicated by the classification of $F_2$ plants. Incomplete dominance of the gene conditioning the resistant reaction in Ricardo was suggested by the presence of plants among the resistant portion of the $F_2$ population and in the segregating $F_3$ progenies of plants that exhibited a moderate degree of brown flecking, whereas all the plants in the homozygous resistant $F_3$ families showed only an occasional fleck. When the resistant plants in the $F_2$ population of the cross Ricardo × Atlas were separated into 2 groups by degree of flecking, there were 185 plants with a 0–1 reaction, 301 plants with a 1+ reaction, and 165 susceptible plants (type 4). This distribution gave a satisfactory fit to the 1:2:1 ratio and confirmed previous observations that the heterozygous plants could be distinguished from the parental type with a fair degree of accuracy.

The observed frequencies of $F_2$ plants with a resistant or susceptible reaction to infection with culture CR3 from the crosses of Ricardo with the tester varieties Goldfoil, Hanna, and Chinerme showed satisfactory agreement with frequency expected with independent segregation of genes conditioning resistance.

Since no plants susceptible to infection with culture CR3 were observed among the progenies from the crosses of Ricardo with Algerian, the gene for resistance in Ricardo is either the same as the $Ml_a$ gene, closely linked, or an allele. However, the phenotypic expression of the genes could be distinguished. The $Ml_a$ gene in Algerian produced a type 0 reaction and was completely dominant in expression, whereas the gene in Ricardo permitted an occasional brown fleck

**Table 2.**—Seedling reaction of parents, $F_2$ plants, or $F_3$ families from crosses of barley varieties Algerian, Ricardo, and Manchuria to infection with culture 061 of *Erysiphe graminis hordei*.

| Generation | Parent or hybrid | Seedling reaction (number) | | | Ratio | Value of P for ratios indicated |
|---|---|---|---|---|---|---|
| | | Resistant | Segregating | Susceptible | | |
| | Algerian | 400[a] | | 6 | | |
| | Ricardo | 0 | | 360[a] | | |
| | Manchuria | 0 | | 654[a] | | |
| $F_3$ | Algerian × Manchuria | 9 | 25 | 16 | 1:2:1 | >.30 |
| $F_2$ | Algerian × Ricardo | 209 | | 73 | 3:1 | >.50 |
| $F_2$ | Ricardo × Manchuria | 0 | | 295 | 0:16 | >.99 |

[a] Parent varieties were included as checks in the tests.

when homozygous, and moderate flecking when heterozygous. A sufficient number of $F_3$ families were grown to reduce to less than 1.0% the maximum amount of crossing over that might have occurred without detection if linkage was involved.

Although the segregation of $F_2$ plants resistant and susceptible to infection with culture CR3 from the cross Ricardo × Psaknon agreed with that expected for independent gene action, there was a deficiency of the susceptible types that would be expected with loose linkage. The recombination value calculated from the data in Table 1 gave a crossover value of 39.4±4.53% between the $Ml_a$ and $Ml_p$ loci. This is in close agreement with the value of 41.5±2.7% reported by Schaller and Briggs (11).

Sixty-six $F_3$ families from the study of the $Ml_k$ gene in Kwan were tested with culture CR3 in addition to the $F_2$ population. Progenies from plants that had the single dominant heterozygotes or the coupling-phase double heterozygote (with close linkage) would segregate in a ratio of 3 resistant to 1 susceptible plant. Of the 66 $F_3$ families observed, 8 were found to be segregating. No homozygous susceptible families were found. The combined estimate of linkage by the method of maximum likelihood from the $F_2$ and $F_3$ data gave a recombination value of 8.8±2.7%. This estimate is in close agreement with 10.4±2.8%, the value reported by Schaller and Briggs (11) for the $Ml_a$ and $Ml_k$ loci.

Table 2 shows the segregation of seedlings from the $F_2$ and $F_3$ generations of crosses between the varieties Algerian, Manchuria, and Ricardo for reaction to infection with culture 061.

The ratio of 1 homozygous resistant to 2 segregating to 1 homozygous susceptible $F_3$ families to infection with culture 061 from the cross of Algerian by Manchuria is the ratio expected if one gene in Algerian conditions resistance to culture 061. The reactions on the plants in the segregating families were similar to reactions on either Algerian or Manchuria, with no intermediate infection types observed. This indicated that the gene in Algerian conditioning resistance to culture 061 was completely dominant.

The frequency of plants resistant and susceptible to infection with culture 061 in the $F_2$ generations from the cross of Algerian by Ricardo fits the 3:1 ratio expected if one gene conditions resistance to culture 061. The gene conditioning resistance to culture 061 would be that in Algerian, since Ricardo was susceptible to this culture.

The 295 $F_2$ plants from the cross of Ricardo by Manchuria were all susceptible to culture 061. This was anticipated, since both varieties in the cross were susceptible to this culture.

**Table 3.**—Comparison of observed seedling reactions of parents, $F_2$ plants, or $F_3$ families from crosses of barley varieties Algerian, Ricardo, and Manchuria to infection with cultures CR3 and 061 of *Erysiphe graminis hordei*.

| Generation | Parent or hybrid | Reaction of parents or ratio of $F_2$ and $F_3$ generations to cultures | | Gene conditioning resistance in variety | |
|---|---|---|---|---|---|
| | | CR3 | 061 | Algerian (CR3 & 061) | Ricardo (CR3) |
| | Algerian | R | R | | |
| | Ricardo | R | S | | |
| | Manchuria | S | S | | |
| $F_3$ | Algerian × Manchuria | 1:2:1 | 1:2:1 | $Ml_a$ & $Ml_a$?[a] | |
| $F_2$ | Ricardo × Manchuria | 1:2:1 | All S | | Ml?[b] |
| $F_2$ and $F_3$ | Algerian × Ricardo | All R | | $Ml_a$ & $Ml_a$?[a] | $Ml_a$?[c] |
| $F_2$ | Algerian × Ricardo | | 3:1 | | $Ml_a$?[d] |

[a] Since all 50 $F_3$ families from this cross were found to react the same to cultures CR3 and 061, the gene in Algerian conditioning resistance to culture 061 is $Ml_a$, allelic to $Ml_a$ or less than 3% crossover units from $Ml_a$.

[b] One unidentified gene conditions resistance in Ricardo to CR3.

[c] Gene in Ricardo conditioning resistance to culture CR3 is $Ml_a$, allelic to $Ml_a$ or less than 1% crossover units from $Ml_a$.

[d] Gene in Ricardo does not condition resistance to culture 061, therefore it is not $Ml_a$ but is allelic to $Ml_a$ or less than 1% crossover units from $Ml_a$.

**Table 4.**—Pathogenicity of parental and progeny cultures from the cross of cultures CR3 and 061 of *Erysiphe graminis hordei* on barley varieties Ricardo and Algerian.

| Parent or hybrid | Pathogenicity of parents and number of progeny cultures | | Expected ratio | Value of P for ratio indicated |
|---|---|---|---|---|
| | Avirulent | Virulent | | |
| On Algerian | | | | |
| CR3 | A | | | |
| 061 | A | | | |
| CR3 × 061 | 24 | 0 | 1:1 | <.01[a] |
| On Ricardo | | | | |
| CR3 | A | | | |
| 061 | | V | | |
| CR3 × 061 | 19 | 16 | 1:1 | >.50[b] |

[a] Genes in cultures CR3 and 061 conditioning avirulence on gene $Ml_a$ in Algerian are identical or less than 4% crossover units apart.

[b] Culture CR3 has gene conditioning avirulence and 061 has gene for virulence complementary to gene $Ml_a3$ in Ricardo.

Table 3 compares the observed seedling reactions of the parents, $F_2$ plants, and $F_3$ families from crosses of varieties Algerian, Ricardo, and Manchuria to infection with cultures CR3 and 061.

Since the 50 $F_3$ families from the cross of Algerian by Manchuria inoculated separately with both cultures CR3 and 061 reacted the same to these cultures, the gene in Algerian that conditions resistance to culture 061 is $Ml_a$, allelic to $Ml_4$, or 3% or less crossover units from $Ml_a$. The results of studies of the inheritance of pathogenicity of the 2 cultures, to be discussed later herein, indicate that the $Ml_a$ gene conditions resistance to both cultures.

The $F_2$ plants from the cross of Ricardo by Manchuria segregated in a ratio of 1 resistant to 2 intermediate to 1 susceptible to infection with culture CR3 but were all susceptible to culture 061. Thus, it is apparent that 1 gene in Ricardo conditions resistance to culture CR3, and that this gene does not condition resistance to culture 061. The relationship of this gene to other known genes conditioning resistance to *Erysiphe graminis* f. sp. *hordei* cannot be determined from these data.

All of the plants from the $F_2$ and $F_3$ generations from the cross of Algerian with Ricardo were found to be resistant to infection with culture CR3, but the $F_2$ plants segregated in a 3:1 ratio with culture 061. This suggests that the 2 varieties have the same gene conditioning resistance to culture CR3. Study of progeny from the cross of Algerian by Manchuria, however, showed that the gene $Ml_a$ in Algerian conditioned resistance to both cultures. Therefore, the gene in Ricardo is not gene $Ml_a$ but a gene less than 1% crossover unit from $Ml_a$, and may be considered allelic to $Ml_a$. This allele in Ricardo has been designated $Ml_a3$.

*Genetics of pathogenicity in the pathogen.*—Table 4 shows the pathogenicity of the parental and progeny cultures from the cross of cultures CR3 and 061 of *Erysiphe graminis* f. sp. *hordei* on the barley varieties Algerian and Ricardo.

Cultures CR3 and 061 were both avirulent on Algerian. Since the 24 progenies from the cross of cultures CR3 and 061 were all avirulent on Algerian, the genes in cultures CR3 and 061 conditioning avirulence on gene $Ml_a$ in Algerian are either identical, allelic, or less than 4% crossover units apart.

Culture CR3 was avirulent and culture 061 virulent on Ricardo. The segregation of avirulent and virulent progeny from the cross of these cultures fits the 1:1 ratio expected if the 2 cultures varied by 1 gene for pathogenicity on Ricardo. The culture CR3 would have the gene $A_a3$ for avirulence and culture 061 the gene $V_a3$ for virulence complementary to the gene $Ml_a3$ in Ricardo.

Table 5 shows the complementary relationship of the genes conditioning reaction in the barley varieties Algerian and Ricardo with the genes for pathogenicity in cultures CR3 and 061. The complementary relationship is valid whether the genes in the host are allelic or different but less than 1% crossover units apart. If the genes are allelic, then Algerian has the $Ml_a$ allele at the A locus and Ricardo the $Ml_a3$ allele at that locus, and both cultures have gene $A_a$ for avirulence complementary to the gene $Ml_a$; but culture CR3 would have the gene $A_a3$ for avirulence and culture 061 the gene $V_a3$ for virulence complementary to gene $Ml_a3$. If the 2 genes are distinct but closely linked, it would not be possible to determine—and would be immaterial—whether the gene $Ml_a3$ was dominant or recessive in the variety Algerian: the 2 genes would be inherited as a unit, and the expression of gene $Ml_a$ would be epistatic to gene $Ml_a3$.

**Discussion and conclusions.**—There is considerable information regarding the genes conditioning resistance to *Erysiphe graminis* f. sp. *hordei* in the varieties Algerian and Ricardo. Both varieties had been resistant to all races or strains of the pathogen studied in North America previous to the isolation of culture 061. The gene in Ricardo that conditions resistance to culture CR3 of race 3 was shown by Moseman and Starling (9) to condition resistance to cultures of 5 other races of the pathogen. Likewise, the gene in Algerian

**Table 5.**—Infection types resulting from the interaction of genes conditioning reaction in barley varieties Algerian and Ricardo and the genes for pathogenicity in *Erysiphe graminis hordei* cultures CR3 and 061.

| Host | Genotype of host if genes are: | | Reaction from inoculation with genotype | |
|---|---|---|---|---|
| | Allelic | Separate | $A_a$ $A_a3$ (culture CR3) | $A_a$ $V_a3$ (culture 061) |
| Algerian | $Ml_aMl_a$ | $Ml_aMl_a$:[a]— | R | R |
| Ricardo | $Ml_a3Ml_a3$ | $ml_aml_a$;$Ml_a3Ml_a3$ | R | S |

[a] With close linkage (less than 1% recombination) it was not possible to determine and immaterial whether the dominant or recessive allele was present.

conditioning resistance to culture CR3 was found to condition resistance to these same cultures (unpublished data). Studies involving progenies from crosses of Algerian and Ricardo had indicated that the gene conditioning resistance in Algerian was completely dominant, whereas the gene conditioning resistance in Ricardo was incompletely dominant. Therefore the genes in the 2 varieties appeared to react differently in the heterozygous condition and probably were different genes.

The following conclusions can be made by applying the complementary gene-for-gene relationship between rust reaction in the host *Linum usitatissimum* L. and pathogenicity in the flax rust fungus *Melampsora lini* (Ehrenb.) Lév., as proposed by Flor (3), to the mildew reaction of *Hordeum vulgare* L. and the barley mildew fungus *Erysiphe graminis* f. sp. *hordei*. Studies of the reaction of barley varieties Algerian and Ricardo and progeny from crosses of these varieties to infection with cultures CR3 and 061 of powdery mildew would not only show the relationship of genes in these varieties conditioning reaction to these cultures but would indicate the differences in genes for pathogenicity in these cultures. Likewise, studies of the pathogenicity of cultures CR3 and 061 and progeny cultures from the cross of these cultures on Algerian and Ricardo would not only show the relationship of the genes for pathogenicity in these 2 cultures on these varieties but would indicate the difference in the genes for reaction in these varieties.

From the inheritance of reaction of progeny from crosses involving Algerian, it was concluded that the gene $Ml_a$ in that variety conditioned resistance to both cultures CR3 and 061. Since no plants susceptible to infection with culture CR3 were observed among the $F_2$ and $F_3$ plants from the cross of Algerian by Ricardo, it could be assumed that the same gene or 2 genes allelic or less than 1% crossover units apart conditioned resistance to culture CR3 in these 2 varieties. When progenies from the cross of these 2 varieties were infected with culture 061, the segregation of resistant and susceptible plants fits the ratio expected if the 2 varieties differed by 1 gene. Previous studies have shown that the gene $Ml_a$ in Algerian conditioned resistance to culture 061; therefore, the gene in Ricardo could not be $Ml_a$ but must be a gene allelic to or less than 1% crossover unit from $Ml_a$. This gene was designated $Ml_a3$. The studies of the reaction of Algerian and Ricardo and progenies from crosses of these varieties to infection with cultures CR3 and 061 showed that gene $Ml_a$ in Algerian conditions resistance to both cultures and gene $Ml_a3$ in Ricardo, allelic to $Ml_a$, conditions resistance to culture CR3 but not to 061. By applying the gene-for-gene hypothesis, both cultures must have the avirulent gene $A_a$ complementary to gene $Ml_a$ in Algerian, and culture CR3 must have the avirulent gene $A_a3$ and culture 061 the virulent gene $V_a3$ complementary to the gene $Ml_a3$ in Ricardo.

From studies of the inheritance of pathogenicity of progenies from the cross of culture CR3 by 061 on the variety Algerian it was concluded that the pathogenicity of those cultures on Algerian was conditioned by the same gene for avirulence. The segregation of avirulent and virulent cultures from the cross of cultures CR3 and 061 on Ricardo fits the ratio expected if one culture had the virulent gene and the other the avirulent gene for pathogenicity on Ricardo. Since culture CR3 was avirulent and culture 061 was virulent, then cultures CR3 and 061 have the avirulent gene $A_a3$ and the virulent gene $V_a3$, respectively, complementary to the $Ml_a3$ gene in Ricardo. The studies of the pathogenicity of cultures CR3 and 061 on Algerian and Ricardo showed that culture CR3 has 2 genes for avirulence, $A_a$ on Algerian and $A_a3$ on Ricardo; and culture 061 has the same gene $A_a$ for avirulence on Algerian and gene $V_a3$ for virulence on Ricardo. By applying the gene-for-gene hypothesis, Algerian has gene $Ml_a$ conditioning resistance complementary to the avirulence gene $A_a$ and either the allele $Ml_a3$ or $ml_a3$ of the gene conditioning the reaction complementary to the gene for avirulence $A_a3$, and Ricardo has the gene $ml_a$ conditioning susceptibility complementary to the virulence gene $A_a$ and the gene $Ml_a3$ conditioning resistance complementary to the gene for virulence $V_a3$.

The genes conditioning pathogenicity on varieties Algerian and Ricardo in cultures CR3 and 061, as indicated by the inheritance of genes conditioning reaction in Algerian and Ricardo on those cultures, were verified by studies of the inheritance of the genes conditioning pathogenicity in those cultures. Studies of the inheritance of genes conditioning pathogenicity in cultures CR3 and 061 on Algerian and Ricardo indicated that different genes conditioned resistance to these cultures, that the gene in Algerian conditioned resistance to both cultures, and that the gene in Ricardo conditioned resistance to CR3 and susceptibility of 061. The evidence for the allelic relationship of the genes conditioning resistance in Algerian and Ricardo obtained by combining the results from studies of genes conditioning the reaction to both cultures CR3 and 061 was supported by determination of the most likely combination of genes conditioning reactions in the host that would be complementary to the genes conditioning pathogenicity in the pathogen.—Plant Industry Station, Beltsville, Maryland, and Department of Agronomy, Agricultural Experiment Station, Davis, California.

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# LOCALIZED ACQUIRED RESISTANCE TO TOBACCO MOSAIC VIRUS

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SUMMARY

Local lesions of tobacco mosaic virus (TMV) on bean stopped growing about 2 days after inoculation, whereas local lesions of TMV on *Nicotiana glutinosa* continued to enlarge. No virus was detected in the tissue beyond the lesion. A zone of unparasitized tissue about 2 mm beyond bean lesions was resistant, but inconsistently so, to a second inoculation with TMV. No such zone was detected when the second inoculation was with alfalfa mosaic virus or tobacco necrosis virus, or when TMV-infected *N. glutinosa* leaves were reinoculated. Although TMV on bean normally caused no symptoms beyond the lesions, 3 types of symptoms beyond the lesions occurred occasionally, and one type was induced at will by appropriate heating of the infected leaves. These symptoms show that the immunized tissue is different in other ways from the normal leaf tissue.

**Introduction.**—Acquired immunity is common in animals but rare in plants, and in plants is confined principally to systemic infections with viruses (4). The present study, stimulated by a report (2) of acquired resistance following localized infection of Dianthus with carnation mosaic virus, has been abstracted (10).

**Methods.**—The method most commonly used to demonstrate acquired resistance was to inoculate the primary leaves of beans (*Phaseolus vulgaris* var. Pinto) in strips, at 10 days from seeding, with a suspension containing 0.1% ground tissue of systemically infected tobacco, and to reinoculate the same leaves 2 days later, but over their entire areas. When infection from the second inoculation was inhibited close to infection from the first inoculation, but not inhibited close to control rubbed areas, acquired resistance is presumed to have been induced by the first infection. The first inoculation on the carborundum-dusted upper leaf surfaces was by means of rubbing with a brush containing the virus suspended in 1% $K_2HPO_4$, and the inoculation was localized in 2 strips on one side of the midrib by means of a steel grid with slits 4 mm wide (Fig. 1). At the same time control strips on the opposite side of the midrib, but also on the upper leaf surface, were rubbed with a suspension of healthy tobacco in phosphate. For convenience the tissue adjacent to the infected tissue is called immunized tissue and the tissue adjacent to the rubbed but not inoculated tissue is called control tissue.

It was desirable to use tissue adjacent to inoculated tissue, and not inoculated tissue itself in these tests, since the mere act of rubbing healthy bean leaves induces marked resistance to subsequent infection (Fig. 1-B).

The criterion of infection was the formation of typical lesions, which on bean were angular to circular, brown necrotic areas about 0.3 mm in diameter that result only from TMV infection. Symptoms to be discussed later, which appeared beyond these typical necrotic areas, are not considered lesions. Infection is not necessarily limited to lesions, but lesions are considered the only practical criterion of infection to be used here.

While reduction in numbers of lesions in strips 4 mm wide as a result of prior inoculation of the adjacent tissue has been the main criterion of acquired resistance, other criteria have been the distance from the first infection to lesions formed by the second inoculation, the size of the lesions resulting from the second inoculation, and the amount of virus formed by the second inoculation. These latter criteria have revealed acquired resistance, but were more laborious to use than the counting of lesions.

**Results.**—*Localized acquired resistance.*—The first trial of localized acquired resistance on beans (July 3, 1952), showed partial acquired resistance, and 118 later trials with about 1400 leaves over 7 years have not yielded a method that is consistently and highly effective. In the last 55 determinations in Berkeley on paired half-leaves, there were 5,011 lesions on the control tissues and 751 on the immunized tissues, or there was 85% control as a result of immunizing inoculation. On all but one of the 55 leaves there were more lesions on the control than on the immunized tissues of the same leaf. Considering that the immunization process had to extend only 2 mm from the lesions to be completely effective by the method used, it is believed that current methods of inducing acquired resistance are relatively ineffective.

The localized resistance appeared specific for both host and virus. No localized resistance was detected in similar tests with TMV on *Nicotiana glutinosa*. The immunized tissue of bean inoculated with TMV was not more resistant than the control tissue to alfalfa mosaic virus or to tobacco necrosis virus, both of which cause local lesions on bean.

Many attempts have been made to improve the degree of acquired resistance. The major variables that have been tested are: age of leaves at time of first (immunizing) inoculation, variety of bean, time from first to second inoculation, season, time of day of first and second inoculation, detached leaves on various substrata, inoculation on upper versus lower surfaces of leaves, and variations in temperature during the test. Although no method was clearly superior to another, the specimen in Fig. 1 shows one of the best cases of acquired resistance observed. This leaf was inoculated in strips at 8 A.M. on August 7, incubated at 31°C in the dark from 4 P.M. August 7 to 4 A.M. on August 9, reinoculated over the entire surface at 6 A.M. on August 9, and left in the greenhouse until the photograph was taken, on August 12. In this case there were 152 lesions in the control tissue between the 2 rubbed but not inoculated areas, and only 15 lesions in similar but immunized tissue between the 2 inoculated strips. In this, as in many trials, the few lesions on the immunized tissue were mostly about half way between the margins of the inoculated areas, or were nearly 2 mm distant from the infected tissue.

*Symptoms beyond the lesions.*—Normally there are no symptoms apparent other than TMV lesions on bean (central part of leaf of Fig. 1). If tissue adjacent to the lesion is immunized by the localized infection, as reported here, it might be possible to

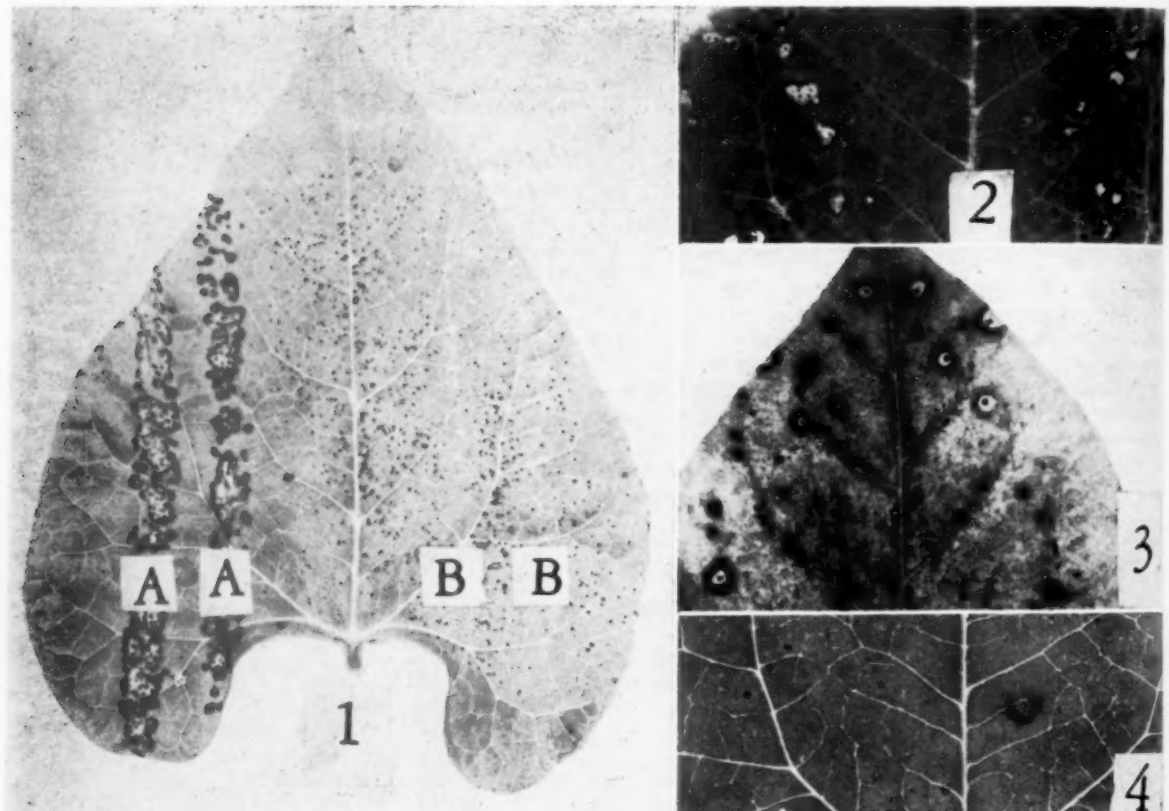


**Fig. 1-4.**—**1)** Acquired resistance to TMV in Pinto bean. A) Infection from localized inoculation at 8:00 A.M., August 7, 1953. These lesions are larger than the others on this leaf, and larger than normal because the plant was incubated 36 hours at 31°C. B) Position of the control rubs on August 7. The entire leaf was inoculated at 7:00 A.M., August 9. Photographed August 12. The comparative number of lesions between the two A strip inoculations and between the two B strip inoculations represents the comparative susceptibility of immunized and control tissues. The few lesions on the B areas indicate the resistance induced by rubbing. **2)** TMV lesions from regular inoculation on July 14, 1955. Photographed July 21. Some of the TMV lesions are surrounded by killed, collapsed areas. Some of the collapsed areas are surrounded or partially surrounded by narrow dark zones. **3)** TMV symptoms induced by heating. This leaf was inoculated in the ordinary way at 3:00 P.M., February 9, 1957, heated 40 seconds at 50°C at 7:00 A.M., February 13, and photographed on February 18. The normal tissue has become chlorotic from heat injury. There is a retention of green color around most of the lesions. Some of the infections, especially those in the distal part of the leaf, show a chlorotic zone of collapsed tissue between the lesion and the ring of green tissues. **4)** Zonate TMV lesion (right center) on leaf inoculated on October 24, 1952. Photographed on October 31. Most of the lesions are normal.

demonstrate some other physiologic differences between the immunized and the normal tissue. Four types of symptoms beyond the lesions have been seen.

The first type observed was a localized protection from smog damage, extending about 1 mm beyond the lesions (13).

A second type (Fig. 2) shows necrosis and collapse of tissue beyond or to one side of the lesions. This type was associated with a marginal breakdown of leaves not associated with virus infection, and for which the cause is unknown, and it appears that TMV lesions have aggravated this breakdown.

The third type of symptom, the most striking, has been induced at will. On bean leaves heated in the early morning to activate virus infection, a variety of symptoms have resulted, but all are considered one type here. On some leaves and with some lesions, the lesions appeared as green islands after the uninfected portion of the leaf had become chlorotic from heat injury (10, and Fig. 3). This type of green-island effect is best known with obligate parasites, such as rusts, where there is known to be a marked accumulation of starch around the lesions. A similar but lesser accumulation of starch has been demonstrated around TMV lesions. Typically the green island was contiguous with the TMV lesion, but in some cases there was a zone of chlorotic collapsed tissue between the lesion and the ring of green tissue. In a typical case the lesion proper was about 0.3 mm in diameter, concentrically surrounded by a chlorotic zone about 1.3 mm in diameter, a dark necrotic zone about 1.5 mm in diameter, and a green zone about 4 mm in diameter. The diameter of the area expressing symptoms was thus about 10 times the diameter of the lesion, or 100 times its area.

A fourth type of symptom was a ring of brownish leaf tissue about 1 mm wide and starting at about 1 mm beyond the lesion (Fig. 4). Neither the brown tissue nor the green tissue it enclosed showed any apparent breakdown. No method of inducing the second or fourth type of symptom is known, and both have been seen only rarely.

*Lack of other changes beyond the lesions.*—The simplest and expected explanation of the localized acquired resistance described here would be that there is virus in the immunized tissue beyond the lesion. Several attempts have failed to confirm this, but it cannot be excluded.

Antibodies were considered. When suspensions of immunized and control tissue were added to TMV suspensions and these then used as inoculum, no significant differences were seen in the numbers of resulting lesions.

When suspensions of immunized and control bean leaf tissue were added to strips of filter paper by the method of Hodes et al (3) and the bases of the strips immersed in a virus suspension, the TMV passed the dried or undried deposits of immunized tissue as well as the deposits of control tissue. Cleared and uncleared suspensions of immunized and control tissues were added to purified and unpurified suspensions of TMV. No reaction was detected immediately or after standing. When the mixtures were heated 10 minutes to 55 or 60°C, the suspensions with immunized tissue sometimes showed a precipitate earlier and in larger amounts than the suspensions of control tissue, but this could not be repeated at will.

*Enlargement of lesions.*—Since acquired resistance was demonstrated with TMV on bean but not on *N. glutinosa*, it is appropriate to compare infections on these 2 hosts. Lesions on both hosts appeared in about 36 hours under prevailing greenhouse conditions. Those on bean reached a diameter of about 0.3 mm and ceased growth at about 2 days, whereas TMV lesions on *N. glutinosa* were about 0.7 mm at 2 days and continued to increase to about 2.7 mm at 7 days (Fig. 5). This might infer that acquired resistance here is associated with a checking of the enlargement of the lesions. In further support of this, TMV lesions on bean at 31°C continued to enlarge and commonly reached a diameter of 2–4 mm in 6 days. In heavily rusted bean leaves at 25° (8) the virus advanced through the tissue at about 1 mm per day and has been detected 6 mm from the point of inoculation. In these conditions where the TMV lesions continued to enlarge, no localized acquired resistance was demonstrated.

**Discussion.**—Localized acquired resistance, which has been so strikingly demonstrated with carnation mosaic virus (2), was quantitatively much weaker with TMV on bean in this study, with TMV on hypersensitive tobacco (5), and with rust infection on bean (11). That localized acquired resistance may be a common phenomenon is indicated by Gäumann (1, p. 280) and Wingard (9, p. 173), but the phenomenon is commonly not mentioned in reviews of acquired immunity (4), and is here considered a minor aspect of disease resistance.

Localized acquired resistance is probably of greatest importance with virus infections, and it may play a part in many situations where it could not be demonstrated by the methods used here. TMV normally

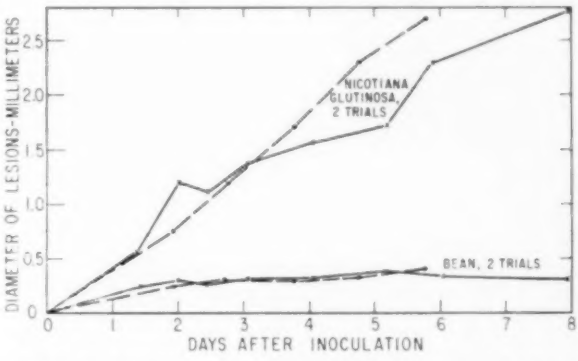


**Fig. 5.**—Growth in diameter of TMV lesions on bean and on *Nicotiana glutinosa*. The cessation of growth of lesions on bean is believed to be a necessary requirement for localized acquired immunity. No localized acquired immunity was detected in *N. glutinosa* when the lesions enlarged at a constant rate.

remains localized in *N. glutinosa* in that, after ordinary mechanical inoculation, the infection does not become systemic at ordinary temperatures, even though the lesions continue to enlarge. That the local-lesion phenomenon in *N. glutinosa* is a type of acquired immunity, even though it could not be demonstrated by the methods of this study, is supported by the fact that if TMV is transmitted to *N. glutinosa* by grafting, it becomes systemic (7, p. 105).

Furthermore if *N. glutinosa* plants are placed at high temperatures after inoculation with TMV, the virus becomes systemic (7). There is thus limited evidence that the phenomenon of localized acquired resistance can be broken down by heat and rust infection in bean and by heat and graft inoculation in *N. glutinosa*.

So far no interpretation of the phenomenon described can be adequately supported. The presence of antibodies has not been established in plants, and there is no clear evidence for antibodies here. The formation of phenolic compounds in the margins of hypersensitive areas of diseased leaves (9) is a plausible explanation, but if this occurs one would expect such immunized tissue to be resistant to a variety of pathogens. But no resistance to alfalfa mosaic virus or to tobacco necrosis virus was detected here. However, Wilson (8) found that TMV-infected tissue was more resistant to rust than was normal tissue, even though rusted tissue was more susceptible to TMV than was normal tissue.—Department of Plant Pathology, University of California, Berkeley.

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# ETIOLOGY AND CHARACTERISTICS OF SWEETPOTATO MOSAIC

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SUMMARY

The mosaic disease of sweetpotatoes was transmitted to sweetpotatoes by fleshy-root core grafts and by sprout grafts but not by sap inoculations to sweetpotato leaves or stems. Mosaic symptoms developed in sprouts produced from core-graft-inoculated sweetpotatoes. Mosaic symptoms did not develop in the aboveground portion of sprout-graft-inoculated plants but only in sprouts from progeny fleshy roots borne by these plants. Tobacco, *Nicotiana tabacum*, plants became infected when inoculated with sap from mosaic-infected fleshy sweetpotato roots but not when inoculated with sap from infected sweetpotato leaves, stems, or fibrous roots. Sap from crushed sweetpotato leaf and stem tissue apparently inactivates this virus. Sweetpotato mosaic virus (SMV) was transmitted from infected tobacco to 13 other plant species. Infection symptoms included mosaic patterns, local necrosis, local non-necrotic lesions, systemic necrosis, and symptomless carriers. The virus was transmitted through grafts from tobacco to sweetpotato fleshy roots. SMV in nonsterile sap from tobacco leaves remained infectious 3 years. This virus remained infectious after being heated 10 minutes at 85°C but not at 90°C. Virus concentration is high in the sap of infected tobacco leaves, as indicated in necrotic lesion production on *N. glutinosa* by dilutions up to $10^{-6}$. Virus concentration in the fleshy roots of infected sweetpotatoes is, however, low and infection is not completely systemic. SMV and tobacco mosaic virus I (TMV) apparently are strains of the same specific virus. Both remain infective for years in nonsterile sap of tobacco, both have a similar thermal inactivation point, and the dilution endpoint for infection in sap from infected tobacco leaves is similar for both. The two viruses are antagonistic, they have common antigens serologically, and the particle size and shape of both are similar in electron microscopy.

A mosaic disease of sweetpotato, *Ipomoea batatas* Lam., in the United States, commonly referred to as sweetpotato mosaic, causes serious disability to infected plants but has been of only slight economic importance because of the few plants infected. Infection incidence has never been more than a trace, and was always scattered in the field. This disease has been observed by the author in Kansas in Nancy Hall or in the mutant varieties Nancy Gold and Red Nancy every year from 1927 to 1959. Notes on the

**Fig. 1.**—Red Nancy sweetpotato plant (left) infected with sweetpotato mosaic by core-graft inoculation. Control plant (right) core-grafted with noninfected fleshy root tissue.

disease have been published since about 1919 (2), but it had not been investigated intensively. The studies reported here were made to learn the cause and characteristics of this disease.

Failures to transmit sweetpotato mosaic were reported by Weimer (7) and by Harter and Weimer (3), who concluded the disease was not caused by a virus. Martin (4) suggested a genetic origin. Rosen (5) reported transmission and symptom development on aboveground plant parts to 2 plants following hypodermic injection of infective sap and to 2 plants after rubbing their leaves with macerated diseased foliage. Elmer (1) reported transmission from infected to noninfected sweetpotato plants through grafting and through sap inoculation from infected fleshy roots to tobacco.

**Symptoms and effects.**—Mosaic-infected sweetpotato plants (Fig. 1) are smaller than normal, upright and bushy, with short internodes and a short vine growth (5). The leaves are strikingly abnormal, being stunted and malformed and developing small, irregular, distinct or diffuse spots or stripes of yellowish or grayish green.

Mosaic-infected Nancy Hall, Nancy Gold, and Red Nancy plants produce smaller and more regularly shaped fleshy roots than do healthy plants. Noninfected roots, in contrast, may have ridged surfaces, may grow to large size, and are characteristically irregular in shape.

Mosaic-infected sprouts were observed developing as frequently from hill-selected as from unselected seed stocks. Instances were noted where sprouts with mosaic symptoms developed in the hotbed from roots produced by plants that lacked mosaic symptoms. Virus transmission had occurred in the field, but symptom expression in the plant had not developed during the same growing season.

Mosaic infection in the plant bed has occasionally been noted either on some or on all sprouts from a mother root. Since infected sprouts appear abnormal, growers usually discard them at planting time, but some may be overlooked and included for field planting. Sprouts from fleshy roots produced by infected mother plants account for all the current-season plants with mosaic symptoms in the field.

**Materials and methods.**—Sweetpotato mosaic virus (SMV) inoculum used in host-range studies consisted of desiccated leaves of tobacco infected directly from sweetpotato fleshy root. Tobacco mosaic virus I (TMV) inoculum consisted of desiccated leaves of tobacco infected with dry leaf-tissue inoculum obtained from the Wisconsin Agricultural Experiment Station and labeled "TMV-severe strain" by Dr. James Johnson. These virus bank inocula were stored dry under refrigeration.

Mosaic transmission attempts were made both by grafting and by inoculations with infective plant sap.

Sprout splice-graft inoculations were made by slicing about 1-in. strips of outer stem tissue from both a mosaic-infected and a noninfected sprout (Fig. 2), tying the sliced surfaces of the 2 sprouts together with rubber strips, and planting each grafted pair in a pot.

Mosaic-infected leaf petioles were grafted into noninfected fleshy roots of Red Nancy. Petioles cut to a V-shape were inserted into V-shape holes punched into the root, after which the injured surfaces were painted with graft emulsion. The graft-inoculated roots were kept in a warm moist chamber about 10 days and planted in moist sand.

Core-graft inoculations to fleshy roots were made by removing a core of tissue with a cork borer from a mosaic-free sweetpotato fleshy root and replacing it

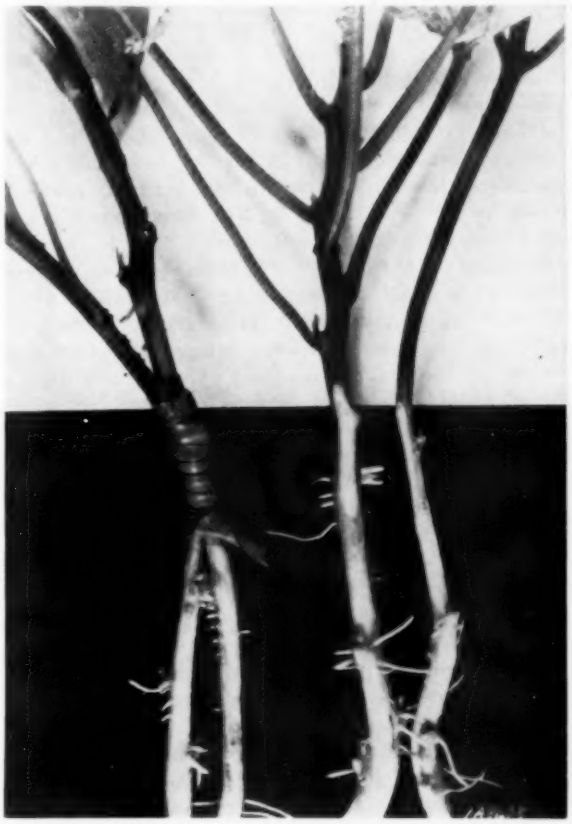

**Fig. 2.**—Technique of splice-grafting sweetpotato sprouts.

with a similar-sized core of mosaic-infected root tissue. Inoculated roots were held about 10 days in a warm moist chamber and then planted in pots.

Plant-sap inoculations were made by first dusting the surfaces to be inoculated with 600-mesh silicon dioxide abrasive powder and then gently rubbing this surface with a cotton swab saturated with sap from the macerated leaves, stems, or roots of the donor plant. Sap from small pieces of infective tissue was used as inoculum after the tissue was macerated in a drop of water on ground glass and then picked up on a moist cotton swab.

Adequate healthy controls were provided throughout, and care was taken to avoid virus contamination of experimental plants. SMV- and TMV-inoculated plants were kept isolated from each other and from noninfected seedling plants. Identity of SMV or TMV was corroborated by differential host reaction to petunia as described below.

**Transmission to sweetpotato.**—Numerous attempts from 1953 to 1957 failed to transmit sweetpotato mosaic by leaf-sap inoculations from infected to noninfected sweetpotato plants. The susceptible varieties Nancy Gold and Red Nancy were used. The inoculations were made by rubbing infective sap onto leaves dusted with silicon dioxide as described above. After plants grew 2–6 months without symptom development, they were discarded. Some of the plants produced small sweetpotatoes. These produced sprouts when planted, but none developed mosaic symptoms.

Mosaic inoculations by splice-grafting healthy with mosaic-infected sprouts were made every year from 1953 to 1958 during March and April. Mosaic symptoms remained evident throughout the summer in the previously infected portions of these plants, but no such symptoms developed in the leaves of the graft-inoculated portions, either in the current growing season or later. In 1955, 50 Red Nancy graft-inoculated plants grown in the field from May to October were taken up and their crowns planted in pots. These plants grew in the greenhouse until May, 1956, when the surviving 43 plants were again grown in the field until October. In no case did mosaic symptoms develop on the graft-inoculated aboveground portions during the 18 months. Mosaic symptoms did, however, occur in sprouts produced by progeny fleshy roots of the graft-inoculated plants.

Progeny sweetpotatoes produced by the mosaic-infected and the inoculated portions of grafted plants were kept separate at harvest time and were planted for sprout production in the greenhouse. Mosaic symptoms developed in some sprouts from fleshy roots borne by both the infected and the inoculated scions. Infection had occurred in the fleshy roots from some of the graft-inoculations, even though symptoms had not developed in the aboveground plant.

Mosaic symptoms did not occur in any of the progeny sweetpotatoes borne by some inoculated plants, even though attachment to the mosaic-infected scion was unbroken. Also, some graft-inoculated plants produced sweetpotatoes, some of which produced mosaic sprouts and others did not. In a 1955 test, for example, with 4 roots from each of 7 proved mosaic-infected Red Nancy plants, sprouts exhibiting mosaic symptoms developed from only 9 of the 28 sweetpotatoes. Some individual roots borne by infected plants produced both mosaic-infected and mosaic-free sprouts. The symptomless sprouts were propagated and appeared not infected because in no case did mosaic symptoms develop in any of them or in sprouts produced by them.

Core-graft inoculations of mosaic-infected fleshy root tissue to noninfected Red Nancy sweeetpotatoes resulted in infection and in development of mosaic symptoms in some, and occasionally in all sprouts produced (Fig. 1). Sprouts in which mosaic symptoms were evident appeared 6 weeks or sooner after core-graft inoculations. Infection symptoms occurred earlier with this method than with sprout-graft inoculations.

*Sweetpotato varietal susceptibility.*—Only the variety Nancy Hall and its mutant varieties, Nancy Gold and Red Nancy, have been observed with symptoms of the sweetpotato mosaic disease in the field. Graft-inoculations were made in April, 1954, to plants of the varieties Kandee, Porto Rico, and Orlis (a mutation variety from Little Stem Jersey) to determine their reaction to this virus. Ten Kandee, 10 Porto Rico, and 20 Orlis sprouts were graft-inoculated with mosaic-infected Red Nancy sprouts. After starting growth in pots, these plants were grown in the field until October, when their progeny roots were harvested. No mosaic symptoms appeared during the summer in the aboveground plant parts that developed from the graft-inoculated scions, but mosaic symptoms remained evident throughout the summer in the infected Red Nancy portions of these plants. The fleshy roots borne by the graft-inoculated Kandee, Porto Rico, and Orlis test plants were planted in the greenhouse and all produced sprouts. Mild mosaic symptoms developed in the leaves of some Kandee sprouts, but no symptoms developed in the Porto Rico or Orlis plants.

Grafts were made between sprouts from mosaic-free Red Nancy sweetpotatoes and from the progeny roots of graft-inoculated symptomless Orlis plants to discover whether Orlis is immune or is a symptomless carrier of this virus. Mosaic symptoms developed on sprouts from some of the progeny roots of the grafted Red Nancy plants, indicating that the Orlis plants though symptomless were actually infected.

**Sweetpotato mosaic inoculations to tobacco and back to sweetpotato.**—Though tobacco, *Nicotiana tabacum* L., is susceptible to many specific mosaic viruses, repeated attempts failed to transmit SMV to this plant through sweetpotato leaf-sap inoculations. Stem-grafting inoculations between stems of mosaic-infected sweetpotato and mosaic-free tobacco plants also failed.

SMV was transmitted to Gold Dollar and Havana 38 tobacco by leaf inoculation of these plants with sap from infected fleshy sweetpotato roots. Such infection was obtained in about 10% of the inoculated plants in 13 attempts in which inoculations were made to 65 tobacco plants. In one attempt, in which 5

**Table 1.**—Results of parallel inoculations of sweetpotato and tobacco mosaic viruses (SMV and TMV) to various hosts.

| Host | SMV infection responses | TMV infection responses |
|---|---|---|
| *Lycopersicon esculentum* L. | mosaic | mosaic |
| *Gomphrena globosa* L. | mosaic | mosaic |
| *Nicotiana tabacum* L. | mosaic | mosaic |
| *N. glutinosa* L. | local necrotic lesions | local necrotic lesions |
| *N. megalosiphon* H. & M. | local necrotic lesions | mosaic, yellowing, necrosis |
| *N. rustica* L. | local necrotic lesions | yellowing, necrosis |
| *Petunia hybrida* Hort. | local necrotic lesions | mosaic |
| *Capsicum annuum* L. | necrosis | mosaic |
| *Solanum melongena* L. | local necrotic lesions | symptomless carrier |
| *Physalis alkekengi* L. | symptomless carrier | symptomless carrier |
| *P. franchetii* Mast. | mild mosaic | (no data) |
| *Physalis* sp. (Mayan husk tomato) | local necrotic lesions | mosaic |
| *Vinca minor* L. | local non-necrotic lesions | local non-necrotic lesions |
| *Datura stramonium* L. | not infected | local necrotic lesions |

tobacco plants were inoculated by rubbing sap from infected fleshy roots on abrasive-dusted leaves. 3 developed mosaic symptoms. In 3 other attempts. 1 each of the 5 inoculated tobacco plants became infected. The experimental plants were grown in a greenhouse at near 80°F. Mosaic symptoms became evident in the tobacco plants in about 4 weeks.

The virus was transmitted back to sweetpotato by grafting mosaic-infected tobacco-leaf petioles into fleshy roots of Red Nancy sweetpotatoes by the method previously described. This resulted in infection in 3 of 5 attempts, in which 5 of 25 inoculated roots produced mosaic sprouts. In one attempt. 3 of the 5 inoculated roots developed sprouts with some exhibiting mosaic symptoms, and in 2 attempts mosaic symptoms developed in sprouts produced by 1 of the 5 inoculated roots.

Sap transmission from SMV-infected tobacco leaves to fleshy roots of Red Nancy was unsuccessful in a test in which the infective sap was rubbed through abrasive powder into the skin of 20 roots. Two successive inoculations were made on the same roots at 4-week intervals. Mosaic symptoms did not develop in sprouts that developed in the greenhouse. later in the plants in the field. or in the sprouts from progeny sweetpotatoes.

**Host-range studies.**—SMV was transmitted to 14 plant species in addition to sweetpotato (Table 1). All of these species are susceptible to TMV. Plant reactions to SMV and TMV were similar in some host plants and widely different in others. The reaction of all host plants to SMV was the same no matter which plant species the infective sap came from.

Confirmation as to whether SMV or TMV was present in infected plants was obtained by differential host-plant reactions of *Petunia hybrida* Hort. and *Nicotiana megalosiphon* H.&M., as described below.

SMV is as easily transmitted from tobacco to tobacco as is TMV. Inoculations of both viruses from tobacco to tobacco resulted in practically 100% infection, and the incubation period of both was about 7 days at temperatures near 80°F.

SMV and TMV both caused systemic infection and mosaic leaf symptoms in Gold Dollar and Havana 38. The first recognizable symptom of SMV infection in Havana 38 leaves is chlorosis at the point of inoculation (Fig. 3). A milder chlorosis of other older leaves was frequent. Immature leaves and leaves produced after infection. developed mosaic symptoms similar to those caused by TMV infection.

Both viruses prevent normal chloroplast production in the developing leaves of infected plants. but SMV also destroyed developed chloroplasts in Havana 38, particularly at the site of infection (Fig. 3).

As noted in Table 1, *Nicotiana glutinosa* L., *N. megalosiphon*, *N. rustica* L., *Petunia hybrida*, *Solanum melongena* L., and *Physalis* sp. (Mayan husk tomato; brought from Guatamala by Dr. I. E. Melhus) are all hypersensitive to SMV. Small, dark necrotic lesions developed in the leaves of these plants at the points of inoculation. Infection symptoms of TMV on *N. megalosiphon*, *N. rustica*, and petunia, by contrast, consist of mosaic leaf symptoms. Petunia, *N. megalosiphon*, and Mayan husk tomato can all be used as differential hosts to confirm the identity of the virus in infected plants. SMV infection produced small, dark necrotic lesions in the leaves of these plants

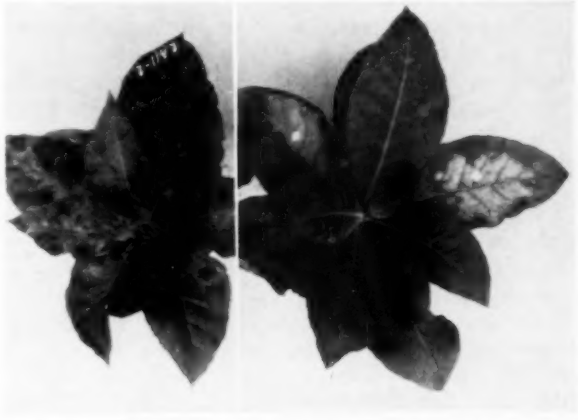

**Fig. 3.**—Symptoms of sweetpotato mosaic virus infection in tobacco. **Left:** Destruction of developed chloroplasts at points of inoculation in Havana 38. **Right:** Inhibition of chloroplast development following infection in Gold Dollar.

within about 3 days, and TMV produced mosaic leaf symptoms in about 10 days.

Pepper, *Capsicum annuum* L. (variety Calwonder), plants developed mosaic leaf symptoms when infected with TMV. When infected with SMV this plant developed tissue necrosis resulting in death of seedling plants and in dropping of older leaves, elongated necrotic streaks along the stem, stunting, barrenness, and sometimes death of older plants. Necrosis was progressive in the tissues but infection did not become systemic, for leaf-sap inoculations to *N. glutinosa* and petunia produced no local necrotic lesions.

Periwinkle, *Vinca minor* L., became infected with both SMV and TMV. Infection symptoms with both consisted of small, circular, whitish, non-necrotic spots with definite margins consisting of collapsed cells. These spots were distributed over the leaf surfaces and continued to appear in newly developing leaves. Under high temperatures, TMV symptoms became masked earlier than SMV symptoms.

**Characteristics of sweetpotato mosaic virus.**—*Longevity in vitro.*—SMV remained infective 3 years in sap extracted from infected tobacco leaves. The sap was extracted and strained through cloth on March 3, 1956, and stored in a stoppered bottle in the greenhouse, which was near 80°F except in the summer, when it occasionally rose to over 120°F. Inoculations made at intervals to March 3, 1959, produced local-lesion infections in both *N. glutinosa* and petunia. Virus concentration of the extracted sap, as indicated by numbers of local necrotic lesions, was reduced an estimated 75% from 1956 to 1959.

*Virus concentration in tobacco.*—Dilutions of SMV from $10^{-2}$ to $10^{-6}$, inclusive, were made with both strained and centrifuged plant sap from infected tobacco leaves. The strained inoculum consisted of sap from macerated infected leaves passed through a cloth filter. A portion of the filtered sap was centrifuged to remove plant debris, including chloroplasts. Inoculations of the 5 sap concentrations replicated 6 times were made to half-leaves and to similar aged leaves of tobacco.

Necrotic lesion infections were most numerous from the $10^{-2}$ dilution, but were still present at $10^{-6}$ following inoculation with both the strained and the centrifuged inoculum. Necrotic lesion counts made after inoculations with the centrifuged sap at the concentrations $10^{-2}$ to $10^{-6}$ were, respectively, 448, 186, 121, 93, and 8.

*Thermal inactivation.*—The temperature at which infectivity of SMV is destroyed was determined by heating extracted sap from infected tobacco leaves and inoculating *N. glutinosa* with the heat-treated inoculum. Ten-minute heat treatments of 3-cc portions of inoculum in glass tubes were made at 5° intervals from 50 to 95°C. After preheating at 35°C, the tubes were immersed 10 minutes in the heated water and then immediately cooled in tap water.

The virus remained infective following the 10-minute treatments at 50–85°C, inclusive, as indicated by development of necrotic lesions in inoculated *N. glutinosa* leaves. No lesions developed from inoculum heated at 90 and 95°C. Thus, the thermal inactivation point for SMV heated 10 minutes is between 85 and 90°C.

**Comparisons of sweetpotato and tobacco mosaic viruses.**—To obtain information on the relation between SMV and TMV, investigations were made of their serological behavior and their antagonism in plant tissues.

*Serological.*—Antiserum produced in animal blood by an antigen, such as a plant virus, reacts not only against that plant virus, but also against other strains of the same specific virus.

Antisera prepared in rabbit blood against TMV were mixed with centrifuged clarified sap from SMV-infected tobacco leaves and, reciprocally, antisera prepared against SMV were mixed with centrifuged sap of TMV-infected tobacco (antisera were prepared by the Department of Plant Pathology, University of Nebraska). Both viruses were diluted 1:10–1:80.

Adding TMV antiserum to SMV sap resulted in a precipitate at 1:10 and 1:20 dilutions. Adding SMV antiserum to TMV antigen resulted in a precipitate at the 1:10 dilution. The reactions against each other's antisera indicate that SMV and TMV are strains of one specific virus.

Sill, Lal, and del Rosario (6) also reported serological studies of SMV and TMV in which antiserum produced against each virus precipitated the other virus. These investigators, through electron-microscope studies, found that the virus particles of SMV and TMV are similar morphologically.

*Antagonism.*—Strains of a specific virus are antagonistic to each other and cannot both infect the same plant cells. Antagonism between two viruses therefore is evidence that these viruses are strains of a specific virus.

Petunia plants systemically infected with TMV and exhibiting severe mosaic leaf symptoms developed no necrotic lesions when the leaves were inoculated with SMV. In one test 10 leaves each of 10 petunia plants infected with TMV and 10 leaves each of 5 noninfected petunia plants were inoculated with SMV by leaf-sap inoculations. Local necrotic lesions developed on none of the 100 leaves of the TMV-infected plants, but developed abundantly on all inoculated leaves of the noninfected controls. No necrotic lesions developed on leaves of TMV-infected *N. megalosiphon* plants inoculated with SMV.

**Discussion.**—The data obtained confirm that the virus of sweetpotato mosaic is a strain of Tobacco Virus I. These 2 viruses are antagonistic within tissues of common host plants, they are similar antigenically, and their particles are similar in size and shape (6). Other like characteristics include similar resistance to thermal inactivation, longevity *in vitro*, and host range.

The effects of SMV and TMV on common host plants are similar for some plant species but widely different in others. In some plants SMV causes more severe effects than does TMV. This is indicated by develop-

ment of necrotic lesions indicating hypersensitivity rather than the milder mosaic symptom reaction that results from TMV infection.

Infection characteristics and reactions of the sweetpotato plant to SMV are distinctive. Transmission of this virus to sweetpotato was accomplished only through graft inoculations. Sap of crushed sweetpotato plant tissue, excepting that of the fleshy roots, contains a substance that is inhibitory to infectivity of this virus, and infection with sap from these plant parts was never successful. The inhibitory substance is not present in sap of fleshy roots, and, through inoculation of fleshy root sap, virus transmission was successful to tobacco. The virus may not be completely systemic in the fleshy roots borne by infected plants. Some but not all sweetpotatoes borne by an infected plant may contain the virus, and only some of the buds of individual fleshy roots may be infected and produce mosaic-infected sprouts.

Aboveground portions of graft-inoculated plants did not exhibit mosaic symptoms when continuously attached 18 months by graft union with an infected scion that continued to exhibit mosaic symptoms. Mosaic symptoms developed only in sprouts produced by progeny fleshy roots of the graft-inoculated plant.

No information was obtained concerning virus concentration in sweetpotato leaves and stems, because sap transmission from these plant parts was never successful. A much lower concentration of SMV occurs in infected fleshy sweetpotato roots than in tobacco plants that were infected with sap from these roots. It may be that the inhibitory substance within the sweet potato plant may also cause the production of a low virus concentration and it may be the cause for the incomplete systemic invasion of sweetpotato fleshy roots.

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# AN APPARENT ASSOCIATION OF HAPLOID LETHAL FACTORS WITH TWO UNIQUE SORUS TYPES OF OAT SMUT

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SUMMARY

Two cases were observed in which lethal factors that prevent haploid growth of oat smut sporidia were associated with sorus type. The lethal expression was operative in 92% of the isolated sporidia observed in a loose smut biotype whereas only 50% of the isolated sporidia of a buff smut biotype reflected the influence of lethal elements.

Systematic study of genetic relationships in the smuts is sometimes hampered by haploid-lethal elements that prevent growth in culture media. In the various species, the effects of haploid lethal deficiencies range from lysis of the entire promycelium to limited budding, or other saprophytic development, of one or more of the primary sporidia produced on a promycelium. The lethal effect expressed by limited budding is characteristic of the oat smuts (*Ustilago avenae* (Pers.) Rost. and *U. kolleri* Wille), in which haploid expressions have been observed in species hybrids (3, 4) and in biotypes within species (2, 4). In some cases up to 98% of the sporidia do not develop, and in others 50% do not (4). In either case a systematic genetic study of specific characters is prevented, because the haploid-lethal expressions in sporidia eliminate some of the potential genetic populations. This problem was encountered in a recent attempt to establish the inheritance pattern for the sorus types in certain biotypes of the oat smuts (6).

The oat smut species produce several distinct morphologic types. *Ustilago avenae* usually produces a brownish, powdery sorus confined to the inflorescence. A variant biotype of this species produces a black, indurate sorus likewise confined to the inflorescence and inherited as a simple recessive in crosses with the brown, powdery type (5). Another biotype of *U. avenae* that produces black, indurate sori in both the inflorescence and its enveloping sheath and leaf tissues (Fig. 1) was recently encountered. The spores of this sorus type were used for an attempted study of its mode of inheritance.

Buff smut, a mutant of *U. kolleri* (4), normally converts all florets of a spikelet into indurate sori that remain enclosed by the outer glumes. Several variations of this sorus type have been encountered. In one type the apical floret is converted into a sorus and the remaining florets are sterile (Fig. 1). An attempt was

made to study the mode of inheritance of this unique character.

**Materials and methods.**—Spores of the two sorus types just described were germinated on standard potato-dextrose agar, and monosporidial isolates were made by standard procedures (1, 5) with a Chambers micromanipulator. Isolated sporidia were transferred to hanging drops of agar medium for convenience in observing further development under low-power magnification.

**Results.**—*Growth in culture.*—Although different degrees of lethal expression were observed among the sporidia of both sorus types, the lethal effect was generally greater in those originating from the flag leaf sorus of the *U. avenae* biotype. Of 308 single primary sporidia isolated in sets of 4 from 77 spores of this sorus type, 283 (92%) were lethal. Only 25 produced culture colonies; 23 of these came from different spores and 2 from one spore. The remainder of the sporidia exhibited lethal effects ranging from immediate inhibition of budding in 43 sporidia to delayed inhibition of budding, as expressed in the production of 2–25 sporidia. At this point budding ceased and lysing occurred. These varying degrees of lethal expression, as exhibited by the four primary sporidia from one spore along with the non-lethal expression of one sporidium, are illustrated in Fig. 2. The effect was immediate on one sporidium, delayed to the first budding on another, and delayed for several generations of budding on a third, whereas the fourth sporidium continued budding indefinitely.

The lesser degree of lethal influence on haploid growth expressed in sporidia from spores of the buff smut biotype was evident in the development of 400 single primary sporidia, representing sets of 4 from 100 spores. Only 215 (54%) of these were lethal, as exhibited by immediate or delayed inhibition of budding. The remaining 45% of the isolated primary sporidia produced normal monosporidial cultures. The pattern of lethal to non-lethal expression in each set of 4 sporidia was 2:2 in the great majority of cases (81 of 100). There were 3 other patterns: all lethal

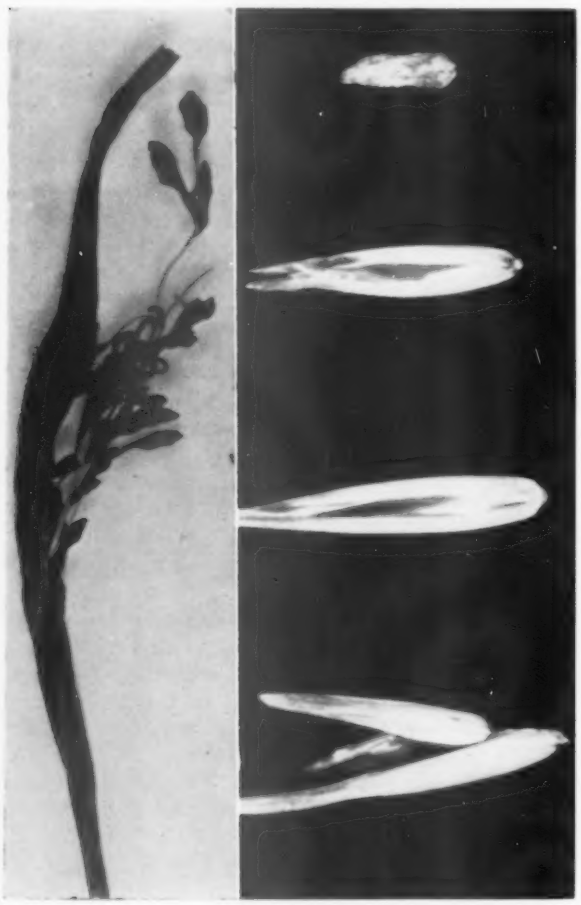

**Fig. 1.**—Oat smut specimens illustrating 2 unique sorus types associated with haploid-lethal deficiencies. **Left:** Anthony oats infected with biotype of *Ustilago avenae* that produces sori in leaf tissues. **Right:** Anthony oats infected with biotype of buff smut that produces sorus in apical floret only. Bottom to top: Entire spikelet and first, second, and third (apical) florets.

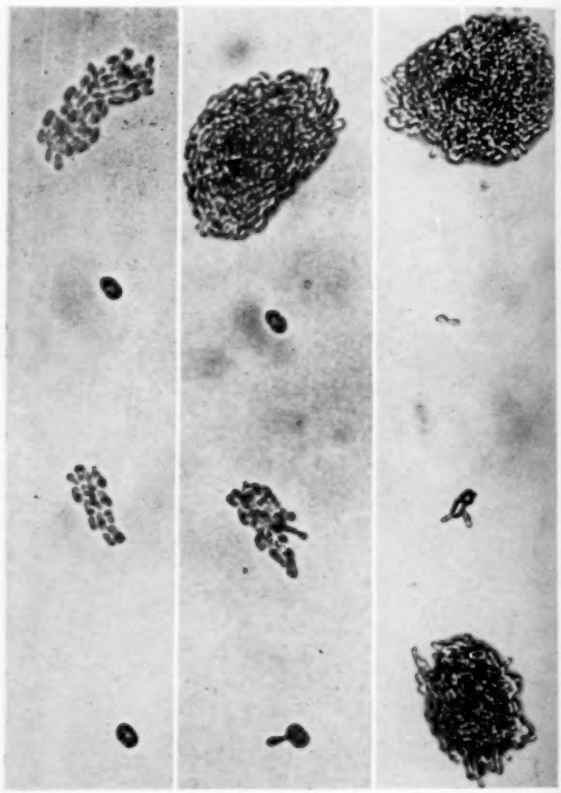

**Fig. 2.**—Photomicrographic illustration of differential lethal effects on growth in culture of two sets of 4 isolated primary sporidia of oat smuts. **Left and center:** Two stages in the development of one set of 4 sporidia of the *Ustilago avenae* biotype that produces sori in the leaf tissues, showing, bottom to top, delayed inhibition of budding of the first two sporidia, immediate inhibition of budding in the third one, and no lethal effect on the fourth (top) one. **Right:** One set of 4 sporidia from a spore of a buff smut biotype that produces sori in the apical florets only, showing the lethal effects on haploid growth of two of the four sporidia.

in 5 sets, none lethal in 3 sets, and 3:1 lethal to non-lethal in 11 sets. The predominant 2:2 pattern is illustrated in Fig. 2.

*Pathogenicity and sex compatibility.*—Pathogenicity of the two sorus types involved in this study obviously is not associated with the lethal influence on growth in culture, since both types can be maintained by spore inoculation. Nor was there any association between the lethal and sex compatibility factors, at least in the *U. avenae* biotype. Both plus and minus compatibility factors were represented in about equal numbers in the 25 monosporidial lines of this biotype obtained from 308 primary sporidia that were isolated. High infection levels were obtained with 10 compatible pairs of these lines, none of which produced sori in the leaf tissues. The significance of these results is not immediately apparent, although a possible linkage is indicated between the haplo-lethal factor and the sorus type involved.

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# THE FEATHERY MOTTLE VIRUS COMPLEX OF SWEETPOTATO

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SUMMARY

Five years of study indicate that sweetpotato feathery mottle is a virus complex of 3 diseases: Yellow dwarf (*Nanus ipomoeae*), internal cork (*Lethum rhizospilum*), and leafspot (*Phyllospilum ipomoeae*). The yellow dwarf component is naturally transmitted in Maryland by the abutilon whitefly (*Trialeurodes abutilonea*) whereas internal cork and leafspot are transmitted by aphids (*Myzus persicae, Macrosiphum solanifolii*, and others). Several United States sources of yellow dwarf virus (Beltsville feathery mottle, California feathery mottle, Georgia mosaic, and New Jersey yellow dwarf) were shown to be graft- and whitefly-transmissible, to produce essentially the same vein-clearing symptoms on indicator plants, and to have the same incubation periods. All the virus isolates of yellow dwarf appear alike, though none is as chlorotic or stunted or injurious as the feathery mottle complex of three viruses.

**Introduction.**—Field diseases and storage rots probably reduce the marketable crop of sweetpotatoes as much as 10%. During the past decade virus diseases that now cause an additional 10% loss have become recognized as an ever-increasing menace. Losses occur in both field and storage. Virus diseases reduce yield and affect quality, and may cause roughness, poor color, and poor shape.

Among the viruses that pose a serious threat to the sweetpotato crop is a virus complex called feathery mottle. It may be considered a virus complex in the light of present findings that feathery mottle as first described may have been a mixture of two or more viruses. This paper clarifies the virus relationships of the feathery mottle complex and describes more fully its relationship to the yellow dwarf vein-clearing phase of the disease.

**Review of literature.**—Feathery mottle was originally described as a new graft-transmissible virosis of sweetpotato (4). The name feathery mottle was suggested because the disease appeared to differ both in symptoms and in methods of transmission from sweetpotato mosaic, described earlier (20, 21). Feathery mottle was then transmitted only by grafting and, because its insect vector was unknown, the virus was given the Latin designation *Flavimacula ipomoeae* sp. nov. The disease was first described in the Russian variety (P.I. 105945), introduced into the United States in 1934 and grown to a limited extent on the Eastern Seaboard.

Elmer (5) recently demonstrated that the sweetpotato mosaic in Kansas is caused by a sweetpotato strain of tobacco mosaic virus.

Summers (25) found in the Ryukyu Islands a dwarf disease of sweetpotato that results in no edible roots being produced on diseased vines. This root-dwarfing characteristic is descriptive of the yellow dwarf type disease elsewhere, whereas the extreme foliage dwarfing in the Island diseases is suggestive of a more severe strain of virus.

Sheffield (22) reported a virus disease of the mosaic type on sweetpotato in Kenya, East Africa. She later found (23) that at least two viruses attack the sweetpotato in East Africa. Virus A, found only in one locality, caused a relatively mild disease and was transmitted by aphids; Virus B, widespread throughout East Africa, existed in a number of strains from mild to severe, and was transmitted by the whitefly, *Bemisia tabaci* (Genn.).

Borders and Ratcliff (2) reported a mosaic of importance on sweetpotato in Georgia, in plant beds and fields on one farm in 1946. The entire planting was condemned and promptly destroyed. No further mosaic was found until 1952, when it appeared in a number of plantings.

In 1957, Daines (3) reported a new virus disease

**Table 1.**—Summary of 5 years of field transmission of the feathery mottle complex by insect vectors.

| Inoculum source | Indicator plants | Transmission of virus symptoms | | |
|---|---|---|---|---|
| | | Leafspot | Internal cork | Yellow dwarf |
| 1955 Russian (PI 105945) | Porto Rico (CF)[a] | + | + | + |
| | Russian (CF) | + | — | + |
| 1956 Russian (PI 105945)[b] | Porto Rico (CF) | + | + | + |
| | Russian (CF) | + | — | + |
| 1957 Russian (PI 105945) | Porto Rico (VF)[c] | + | + | + |
| | La. XXI (VF) | + | + | + |
| 1958 Yellow dwarf in La. XXI | Porto Rico (VF) | — | — | + |
| | La. XXI (VF) | — | — | + |
| 1959 Yellow dwarf in Porto Rico, La. I, La. VII, and La. XXI | Porto Rico (VF)[d] | ± | — | + |
| | La. I (VF) | ± | — | + |
| | La. IX (VF) | ± | — | + |
| | La. XXI (VF) | ± | — | + |
| | Yellow Jersey (VF) | — | — | — |
| | Nemagold (VF) | — | — | — |

[a] CF = cork-free.
[b] The Russian source was grafted onto virus-free Porto Rico, which was planted as source along with the initial inoculum in Russian.
[c] VF = virus-free (freedom from all virus symptoms, including leaf-spots). Seedlings La. I, VII, IX, and XXI have been maintained free of virus.
[d] To find out what effect it would have on Yellow dwarf, Row 7 was planted to Porto Rico containing only leafspot (strain #4). ± equals trace of leafspot showing in "flush of growth."

on sweetpotato in New Jersey. It occurred on the Georgia Red variety, sprouts of which had come from Georgia in 1956. This disease was called yellow dwarf (11).

Martin (19) published an annotated review on mosaic and similar diseases of sweetpotato reported from around the world.

Girardeau (6) demonstrated that the sweetpotato whitefly, *Bemisia inconspicua* (Quaint.) (syn. *B. tabaci*), is a vector of sweetpotato mosaic in south Georgia and is the same vector as that in Africa and Israel (18).

Webb (26) and Webb and Larson (27), using Doolittle's feathery mottle in studies in Wisconsin, demonstrated aphid transmission of certain components of the feathery-mottle complex. They also indicated a close relationship between the aphid-transmitted component and a naturally occurring virus found on sweetpotato in Wisconsin and Louisiana.

Stubbs and McLean (24), using a California isolate of feathery mottle, demonstrated aphid transmission of certain of its components on the Yellow Jersey variety and on morning glories. The California materials might have contained the same aphid-transmissible components as the original feathery mottle at Beltsville.

Hildebrand and Smith (15, 16) demonstrated aphid transmission of the leafspot components of the feathery mottle virus complex, and at the same time found that the yellow dwarf component was not aphid-transmissible. Natural transmission of sweetpotato feathery mottle under field conditions was reported later at Beltsville, Maryland (10). Hildebrand (12) reported a greatly increased natural transmission of yellow dwarf virus in 1957, the result of a climatic aberration, that forced the abutilon whitefly, *Trialeurodes abutilonea* (Hald.), to leave the defoliated weed host earlier than usual to feed and breed on sweetpotatoes. Current-season symptoms of yellow dwarf disease were observed for the first time at Beltsville in 1957 (10), something that occurs every year in East Africa, where *Bemisia tabaci* is endemic (23).

Hildebrand (13) reported on the biology of the abutilon whitefly, *T. abutilonea*, the natural vector of the yellow dwarf component of the sweetpotato feathery mottle virus complex in Maryland. Under caged conditions in the greenhouse or laboratory, the adult survived 3 days or less, which is quite different from the longevity of about 5 weeks reported by Avidov (1) for *Bemisia tabaci* in Israel.

Loebenstein and Harpaz (18) concluded that at least three different viruses affect sweetpotatoes in Israel: the vein-clearing virus, which is transmitted by *Bemisia tabaci*; the ringspot virus, which is aphid transmitted; and the leaf pucker virus. Their claim that these viruses are distinct from those reported in other countries has not been substantiated.

**Materials and methods.**—*Field studies.*—For the field studies, an isolated plot (40×120 ft) called West Nut (WN) was used for 4 years in succession (1955–58). Once in 3 years, aldrin was applied to control soil insects. A similar plot (70×80 ft) in the same immediate neighborhood was planted in 1959. It was about 500 ft from other sweetpotatoes, and separated from them by a planting of corn. The original plot contained seven 50-plant rows 4 ft apart, with the plants 2 ft apart in rows. The 1959 plot contained thirteen 30-plant rows.

To determine whether natural transmission had occurred, single roots from each hill were bedded in sand in the greenhouse following curing, and 3–5 sprouts were planted in 4-in. pots and carried

through the "flush of growth" technique (9). Any sweetpotato virus, regardless of identity, was thereby usually revealed within one month of potting. The other roots were held 6 months in warm storage (75°F, 85% RH) and then sliced.

The 1955 planting consisted of 2 rows of feathery-mottle-diseased Russian variety (P.I. 105945), 4 rows of cork- and feathery-mottle-free Russian, and 1 row of cork-free Porto Rico. The 1956 planting was the same except that 1 row of Porto Rico with feathery mottle was substituted for 1 row of the Russian source.

In 1957, totally virus-free plants were substituted for the feathery-mottle- and cork-free Russian and Porto Rico varieties that had previously contained some leafspot virus. The 5 healthy rows now consisted of 2 rows of virus-free Porto Rico and 3 rows of virus-free La. XXI seedling. As in 1956, the feathery mottle virus source was Row 2 (diseased Porto Rico) and Row 6 (diseased Russian). When none of the numerous winged insects tested between 1954 and 1957 were found to be vectors of feathery mottle, it was suspected that the natural vector was possibly harbored on a weed growing in the immediate vicinity. Therefore, weeds were allowed to grow on the border of the planting for the first time. Support for this hypothesis came from the natural transmission each year (Table 1).

The 1958 planting was essentially the same as in 1957 except for the addition of Row 8 (La. IX seedling) and the substitution of only the yellow dwarf virus component for feathery mottle as the virus source.

The 1959 planting was thirteen 30-plant rows—8 of virus-free selections (2 Porto Rico, 2 La. I, 2 La. IX, 2 La. XXI), 1 of Old Porto Rico that was cork-free and practically free of leafspot, and 4 yellow-dwarf-diseased rows of Porto Rico, La. I., La. VII, and La. XXI.

*Greenhouse and laboratory studies.*—The indoor studies were correlated with the field work and centered on separation of the feathery mottle virus complex into the ultimate or specific components.

Of the numerous morning glory plants grafted during 1954–58, only *Ipomoea alba* produced a systemic vein-clearing (Fig. 1) although both Scarlett O'Hara

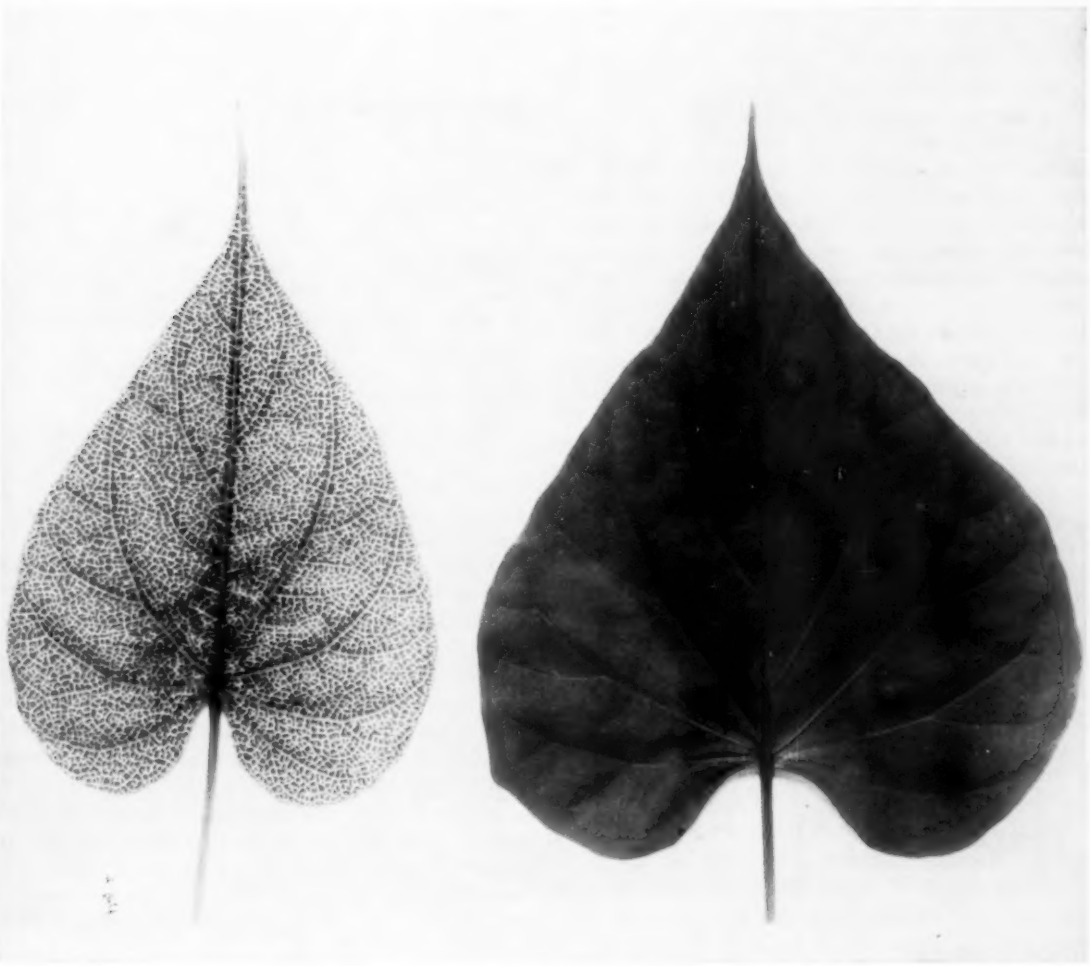

**Fig. 1.—Left:** Vein-clearing symptoms on *Ipomoea alba* morning glory resulting from graft transmission of feathery mottle from Russia. **Right:** Normal leaf. Phot. June 6, 1955.

morning glory and *Quamoclit angulata* proved more generally satisfactory as indexing hosts. Graft-transmission was used exclusively until the discovery of the vector role of the abutilon whitefly, in late 1957 (12, 13). A good graft union was essential for transmission of the systemic vein-clearing yellow dwarf. The chip-bud graft was practically worthless for transmission of yellow dwarf, except when the occasional good union was formed. Great care was exercised to eliminate cross-contamination in the greenhouse by excluding vectors, such as men using tobacco, by fumigation at weekly intervals, by steam sterilization of pots and soil, and by other sanitary measures.

The greenhouse problem of greatest importance was the development of experimental plants that were virus-free. To this end, seeds of diseased sweetpotatoes and morning glories were produced, germinated, and grown, and by the end of the 1956 studies were all found to be virus-free. Seed was obtained from open-pollinated sweetpotatoes produced in Louisiana. About 300 seedlings were started early in 1956, of which 57 formed the basis for this study. The 57 seedlings were graft-inoculated in duplicate with tip scions from feathery mottle and internal cork syndromes and included the same number of seedling checks.

**Results and discussion.**—1955 *studies.*—There was 8% natural transmission of the feathery mottle complex in 1955, as determined by the "flush of growth" technique. Of equal importance was the spread of internal cork and leafspot symptoms in the experimental planting, presumably from the feathery mottle source. When the roots were sliced after 6 months of warm storage, the Russian variety was cork-free whereas about 20% of the Porto Rico roots showed typical lesions of internal cork. What was the source of the cork virus in Porto Rico? It was noted that both the Russian and Porto Rico varieties showed foliage symptoms during the growing season, some of which appeared typical of internal cork. Since the cork-free Porto Rico plants also carried a leafspot there was a dual source of inoculum for the latter component. Finally, as to source of the cork virus, the Russian variety appeared to be the most likely possibility, even though it showed no root lesions.

The most important result in 1955 (15, 16) was the evidence that the aphid vector of the leafspot viruses of sweetpotato was not the vector of the systemic yellow-dwarf component of feathery mottle.

*1956 studies.*—The 1956 studies largely repeated and confirmed the field and laboratory work of 1955, but with some differences. The "flush of growth" technique demonstrated about 6% natural transmission of feathery mottle to both the Porto Rico and Russian varieties. Another important observation was the mortality of about 20% in feathery-mottle-diseased Porto Rico, compared to 2% in diseased Russian. The graft-inoculation study of the 57 Porto Rico seedlings revealed that an outstanding indicator for the yellow dwarf component of feathery mottle was La. XXI, which was highly resistant to internal cork and the leafspot viruses.

All the roots were saved from Row 1 of the 1956 planting containing cork-free Porto Rico. From each hill, single medium-size roots, sliced after about 5 months of warm storage, showed 25% natural transmission of internal cork. In Row 1 the number of roots was 4–14 per hill. At the final slicing (September 10, 1957, about 6 months later), 46% of the hills contained some roots with cork lesions. Only 2 hills had all their roots affected. Only 1 hill of 11 roots had only a single root affected. Thus in the 23 hills with root lesions, 96 roots had lesions and 96 roots did not. A row of the cork-free Russian seedling handled in the same way contained no root lesions, and was evidence of this variety's high resistance or immunity to root cork.

The doubling in incidence of root lesions between the 5- and 11-month storage intervals can be confusing. However, the first figure is based on slicing a single medium-sized root and the latter on slicing 3–13 of the remaining roots in the particular hills 6 months later. During that time lesion size definitely increases but very few new lesions appear. To demonstrate how root size is related to the incidence of root lesions, the percentage incidence of root lesions from the largest to the smallest roots in the hills was: 1) 42; 2) 30; 3) 28; 4) 26; 5) 27; 6) 37; 7) 22; 8) 19; 9)8; 10) 25; 11) 12; and 12) 0. Recalling that the medium-size root, or about No. 4 was employed for the first slicing, it will be noted that the respective percentages of cork incidence were 25 and 26%.

*1957 studies.*—Natural transmission of feathery mottle in 1955 and 1956 and the failure to locate a natural, winged vector by sweeping, suggested that the true vector might be harbored on a native plant. Therefore, weeds were allowed to grow undisturbed on the border of the 1957 planting.

The new departure in the 1957 planting was the substitution of totally virus-free plants for the cork-free Russian and Porto Rico varieties previously used. By this arrangement the only virus source was the feathery mottle inoculum present in the Porto Rico and Russian varieties. Thus, by the end of the 1957 season the natural agents of virus transmission had spread the feathery mottle virus mixture to virus-free plants and given rise to some 15% internal cork, 30% yellow dwarf, and over 90% leafspot, all coming from the one source. At digging time no symptoms were in evidence except leafspot, which occurred in about one-half of the hills. When the roots were cured and put through the "flush of growth" (9) all hills showed leafspot whereas 13 hills, or 31%, showed feathery mottle, or yellow dwarf in mixture with leafspot and internal cork. When slicing in April, 1958, after 6 months' warm storage, 6 hills, or 15%, had root lesions of internal cork and of these one-half had feathery-mottle.

In scouting for the natural vector, it was noted in early July that the Indian Mallow weed (*Abutilon*

*theophrasti* Medic.) was lightly populated by a whitefly. The hot, dry weather in August favored whitefly infestation to build up to the point of defoliating the weed, and the adult insect therefore moved onto the sweetpotato to breed. Abundant rain in late August and an unusually warm September stimulated the drouth-stunted sweetpotato to abundant growth, accompanied by current-season symptoms by mid-September, with about 10% of the virus-free plants showing symptoms before the end of the growing season.

During 1957, numerous grafting experiments were run to determine the minimum incubation period of yellow dwarf. Ordinary grafting demonstrated a tendency of the virus to become imprisoned in sweetpotato and unable to express symptoms during the current growing season. Previously, graft-inoculation studies (7) on a woody-plant virus, which had the same delaying tendency, demonstrated how "shock treatments" stimulated a "spurt of growth" by the plant and symptom production on the first new growth. By synchronizing graft inoculation with the stimulation of new growth on the suscept, the incubation period for yellow dwarf was shortened to 8–14 days. When the feathery mottle virus mixture was the graft inoculum, yellow dwarf symptoms appeared as promptly as when alone. Leafspot symptoms of the internal cork syndrome appeared 1–2 weeks later, on older leaves 5–7 nodes below the shoot tip that displayed the dwarf symptoms.

In the field, on July 15 there was only a trace of the leafspots characteristic of internal cork and leafspot viruses, whereas by mid-September over 50% of the plants showed similar symptoms and about 10% showed current-season symptoms of yellow dwarf. Immediately after harvest and curing, the "flush of growth" technique revealed the complete story for yellow dwarf (31%) and for leafspot (100%). After 6 months of warm storage (about April 16, 1958) 14% of the virus-free Porto Rico had root lesions when sliced.

*1958 studies.*—The biology of the abutilon whitefly was studied under both controlled and field conditions to establish the feeding and breeding habits and other peculiarities of this insect as a vector type (12, 13). The micrurgical aspect of inoculation and infection (14) was particularly pertinent.

Preliminary unpublished micrurgical observations established the fact that "crawlers" from eggs that hatched on healthy plants could be transferred by semi-micrurgical methods to the undersides of diseased leaves, where they would grow to maturity and emerge as viruliferous adults, ready to be employed in transmission studies. When the crawler comes to rest it soon fastens itself permanently to the leaf, by stylet and wax, with the simple stylet *in situ* from the remaining larval stages. Emerging from the pupal case about 12 days later, the whitefly bears a compound stylet. The biology of the greenhouse whitefly *Trialeurodes vaporariorum* (Westw.) is morphologically very similar to that of the abutilon whitefly, but it proved to be a nonvector of yellow dwarf.

During the 1958 studies on the incubation period of the yellow dwarf virus, numerous graft and whitefly experiments were successful. The minimum incubation period for graft transmission was 10–14 days on both sweetpotatoes and morning glories. The minimum for whitefly inoculation was 11–15 days on morning glory suscepts (Scarlett O'Hara (*Ipomoea nil*), *I. alba*, and *Quamoclit angulata*), and usually somewhat longer on sweetpotatoes.

In the tests comparing graft versus whitefly transmission, the abutilon whitefly was able to pick up and transmit the yellow dwarf virus from all sources tested —feathery mottle in the Russian and Porto Rico varieties; feathery mottle in the Yellow Jersey from California; and mosaic in Georgia Red from Georgia and New Jersey. There was a difference in symptoms, however, when graft transmission was employed, because grafting transmitted all the components of the feathery mottle virus complex. For example, grafting induced a chlorosis and spotting on top of vein clearing, which latter is characteristic of the whitefly mode. Such other variations as were found within and between the two modes of virus transmission are attributed to how well the two basic essentials, spurt of growth and virus presence, are synchronized. With either element out of synchrony, the virus is usually translocated down to the roots, where it is imprisoned for the rest of the growing season. Hence, no current-season symptoms are evident. Even though carefully conducted, the whitefly transmission experiments were less than 30% in synchrony, whereas the grafting mode was usually much higher, up to 100% when the best sweetpotato indicator (La. XXI) was used. Some indicators were largely useless because of the incompatibility between their stocks and the scions bearing the virus inoculum.

The weather was so favorable for weed growth in 1958 that the abutilon whitefly, though abundant, largely avoided the sweetpotato plant as a feeding ground, and its natural transmission in the field that year was less than 5%, compared to 31% in 1957, when drouth defoliated the weed. When Row 1 of virus-free Porto Rico was sliced after about 7 months' warm storage, no internal cork was recorded. This is evidence that the yellow dwarf virus, unlike the feathery mottle complex, is free of cork virus.

*1959 studies.*—The 1959 studies demonstrated natural transmission of yellow dwarf virus by the abutilon whitefly. Weather conditions in 1959, as in 1958, were favorable for the mallow weed. With essentially the same conditions prevailing again, the vector was tricked by cutting or breaking down its weed host in late August, to simulate the 1957 drouth, and force the whitefly to breed on sweetpotatoes so as to pick up virus and become viruliferous. This stratagem was rewarding because it gave 21% natural transmission to La. XXI, the best indicator host, compared to 10% or less for Porto Rico and the others.

**Table 2.**—The characteristics and the Latin binomials proposed for the viruses present in and separated from feathery mottle.

| | Yellow dwarf | Internal cork (strain #1) | Leafspot (vein-banding strain #4) |
|---|---|---|---|
| Latin name of virus* | *Nanus ipomoeae* | *Lethum rhizospilum* | *Phyllospilum ipomoeae* |
| Host reaction | Vein-clearing and dwarfing of foliage and roots | Spotting of foliage and necrotic root lesions | Leafspots |
| Transmission | Whiteflies and grafting | Aphids, grafting, and micromechanical; mechanical aided by cysteine | Aphids, grafting and micromechanical; also mechanical |
| Properties | Unknown | TDP 58–62°C | TDP 56–58°C |
| Distribution | Limited | Widespread | Widespread |

* A nonspecific Latin binomial is proposed for the common name of the disease instead of the virus because this entity is definitely not a microorganism. For precedent, see use of this terminology by Hildebrand (8), in which sour cherry yellows was called *Chlorogenus cerasae* and ringspot was called *Annulus cerasae*.

Rapid transmission of most systemic viruses is conditioned by having the host plant at the best growth status for virus entry and multiplication. The humid weather of 1959 greatly reduced the field population of the abutilon whitefly, and also made most difficult its production on sweetpotatoes in the greenhouse cages. There was consequently a high insect mortality, apparently due to fungus parasites, in both greenhouse and field, which may largely account for the lack of current-season symptoms in both.

By sharpening the time and amount of shock pruning for producing the best growth status of the host, the minimum incubation period of the Yellow Dwarf virus was reduced from 10 to 8 days in grafting transmission.

Substituting yellow dwarf for feathery mottle in the 1958 and 1959 studies produced an unexpected effect on the Porto Rico variety in particular. Instead of severe dwarfing and death, as occurred in 20% of the plants in 1956, all of the Porto Rico plants survived by virtue of possessing more vitality and growth. In other words, the devitalizing effect from the yellow dwarf component alone was much less than when the 3 components are combined, as in feathery mottle. Hence, the milder effects of the components individually appear to be additive when combined, and much more severe. The fan leaf symptom on Porto Rico, so characteristic of feathery mottle infection, was much less in evidence for yellow dwarf alone.

**Conclusions.**—This study has established that feathery mottle virosis is a virus mixture of three components or viruses—1) yellow dwarf, 2) internal cork, and 3) leafspot—within each of which there appear to be strains. On the basis of symptoms and certain other characteristics, the three viruses appear to be distinct from one another, when separated and studied under controlled conditions in the greenhouse. The whitefly picks up and transmits only yellow dwarf virus in the greenhouse or field whether in pure culture or in the feathery mottle virus complex (Table 1, 1958 data). The aphid picks up and transmits the leafspot viruses of the internal cork syndrome, of which the leafspot component may occur in pure culture in nature. Up to now the internal cork virus has always been found associated with leafspot virus. Table 2 gives their characteristics and the Latin binomials proposed for their designation.

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# PATHOGENICITY AND MORPHOLOGY OF SOME LEGUMINICOLOUS AND RELATED SPECIES OF STEMPHYLIUM

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SUMMARY

Host range and morphological characteristics of 7 species and 4 unidentified isolates of *Stemphylium* from various hosts and locations were compared. Of the species tested for pathogenicity, *S. botryosum* had the broadest host range. *S. sarcinaeforme* infected red clover, Ladino white clover, alfalfa, and white sweet clover. Three isolates of *S. loti* were equally pathogenic on birdsfoot trefoil and blue lupine. *S. trifolii* attacked Ladino white clover more severely than red clover and alfalfa. Isolates of *S. floridanum* and *S. solani* from tomato attacked only tomato, whereas *S. solani* from blue lupine attacked blue lupine severely and tomato slightly. Of 2 unidentified *Stemphylium* isolates from crown vetch, 1 was large-spored and morphologically similar to *S. solani*, and infected crown vetch and tomato, whereas the other was small-spored and nonpathogenic to all plants inoculated. *S. callistephi* and 2 unidentified isolates from Ladino white clover were nonpathogenic to plant species inoculated. The unidentified isolates from Ladino white clover and crown vetch differed morphologically, culturally, or in host range from known species of *Stemphylium*, and are possibly new forms or species. Differences in pathogenicity within species of *S. botryosum* and *S. solani* are possibly due to pathogenic specialization.

In June, 1957, 2 unidentified isolates of *Stemphylium* Wallr. were obtained from crown vetch (*Coronilla varia* L.) in central Pennsylvania (1). One isolate produced large conidia similar to those of *S. solani* G. F. Weber as reported on tomato and lupine (3). The other produced small conidia somewhat similar in morphology to conidia of *S. botryosum* Wallr. and to those of 2 unidentified isolates recently obtained from Ladino white clover (*Trifolium repens* L.). The small-spored isolates from crown vetch and white

**Table 1.** Spore size and morphology of 20 *Stemphylium* isolates grown on V8-juice agar.

| Fungus | Original host | Conidial Size: Range (μ) | Conidial Size: Average (μ)* | Conidium wall | Perithecia (P) or sclerotial bodies (SB) |
|---|---|---|---|---|---|
| *S. botryosum* | Alfalfa | 21–36 × 16–25 | 30 × 20 | echinulate | P |
| | Red clover | 20–36 × 15–23 | 27 × 18 | echinulate | P |
| | Blue lupine | 24–38 × 15–23 | 28 × 18 | echinulate | P |
| | Fescue | 21–37 × 16–28 | 30 × 22 | echinulate | P |
| *S.* sp. | Ladino | 20–34 × 14–21 | 27 × 17 | rough | SB |
| | Ladino | 21–37 × 16–26 | 30 × 20 | rough | SB |
| *S.* sp. (small-spored) | Crown vetch | 18–29 × 12–21 | 23 × 17 | rough | P |
| | Crown vetch | 17–27 × 14–18 | 22 × 15 | rough | P |
| *S. sarcinaeforme* | Red clover | 27–45 × 23–32 | 35 × 27 | smooth | none |
| | Ladino | 28–50 × 24–33 | 35 × 27 | smooth | none |
| | Sweet clover | 25–41 × 18–28 | 33 × 24 | smooth | none |
| *S. loti* | B. trefoil | 25–40 × 18–27 | 32 × 23 | smooth | SB |
| *S. trifolii* | Ladino | 24–40 × 12–18 | 30 × 15 | smooth | SB |
| *S. solani* | Tomato | 33–68 × 16–21 | 52 × 19 | smooth | none |
| | Blue lupine | 40–68 × 15–21 | 53 × 17 | smooth | none |
| | Blue lupine | 39–56 × 17–22 | 45 × 19 | smooth | none |
| *S.* sp. (large-spored) | Crown vetch | 42–63 × 16–23 | 53 × 18 | smooth | none |
| | Crown vetch | 35–62 × 16–25 | 50 × 20 | smooth | none |
| *S. floridanum* | Tomato | 38–59 × 15–19 | 48 × 16 | verrucose | none |
| *S. callistephi* | China aster | 47–81 × 18–32 | 56 × 20 | slightly rough | none |

* Based on measurement of 50 conidia.

clover were not associated with any definite lesions. Investigation was made to determine the comparative host range of several leguminicolous and related isolates and species of *Stemphylium* and to compare them culturally.

**Materials and methods.**—Stock cultures of *Stemphylium* spp. were maintained in test tubes of sterile soil. For host-range and morphological studies, particles from soil cultures were scattered over V8-juice agar in Petri plates and the cultures incubated in the laboratory for 7 days at 18–24°C. For inoculations in the greenhouse, conidia and some mycelium from 3 Petridish cultures were scraped from the agar surface into a liter of distilled water to which one drop of Tween

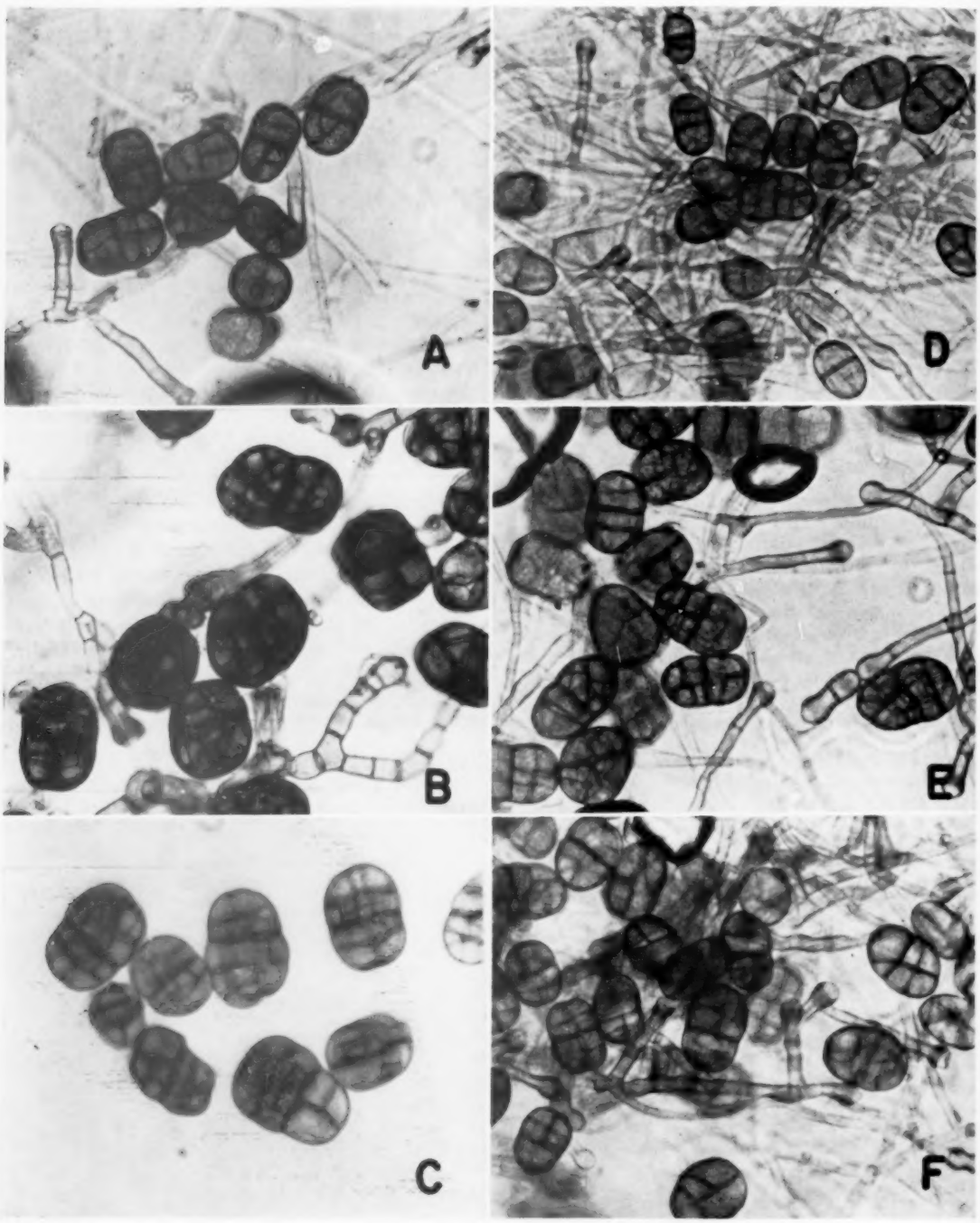


**Fig. 1.**—Conidia of leguminicolous species of *Stemphylium* grown on V8-juice agar. **A)** *S. botryosum* from alfalfa; **B)** *S. sarcinaeforme* from red clover; **C)** *S. loti* from birdsfoot trefoil; **D)** *S.* sp. from crown vetch; **E)** *S.* sp. from Ladino white clover (N.J.); **F)** *S.* sp. from Ladino white clover (N.H.). (×500).

80 was added before comminution in a Waring blender. The following 36 isolates were used in host-range studies: 1) 8 isolates of *S. botryosum* collected in 5 states from alfalfa (*Medicago sativa* L.), red clover (*Trifolium pratense* L.), blue lupine (*Lupinus angustifolius* L.), and tall fescue (*Festuca arundinacea* Schreb.); 2) 4 unidentified isolates of *Stemphylium* from Ladino white clover (2 states); 3) 3 isolates of an unidentified small-spored *Stemphylium* and 3 isolates of an unidentified large-spored *Stemphylium* from crown vetch; 4) 4 isolates of *S. sarcinaeforme* (Cav.) Wiltsh. collected in 3 states from red clover, Ladino white clover, and white sweet clover (*Melilotus alba* Desr.); 5) 3 isolates of *S. loti* Graham from birdsfoot trefoil (*Lotus corniculatus* L.) collected in 3 states; 6) 2 isolates of *S. solani* from tomato (*Lycopersicon*

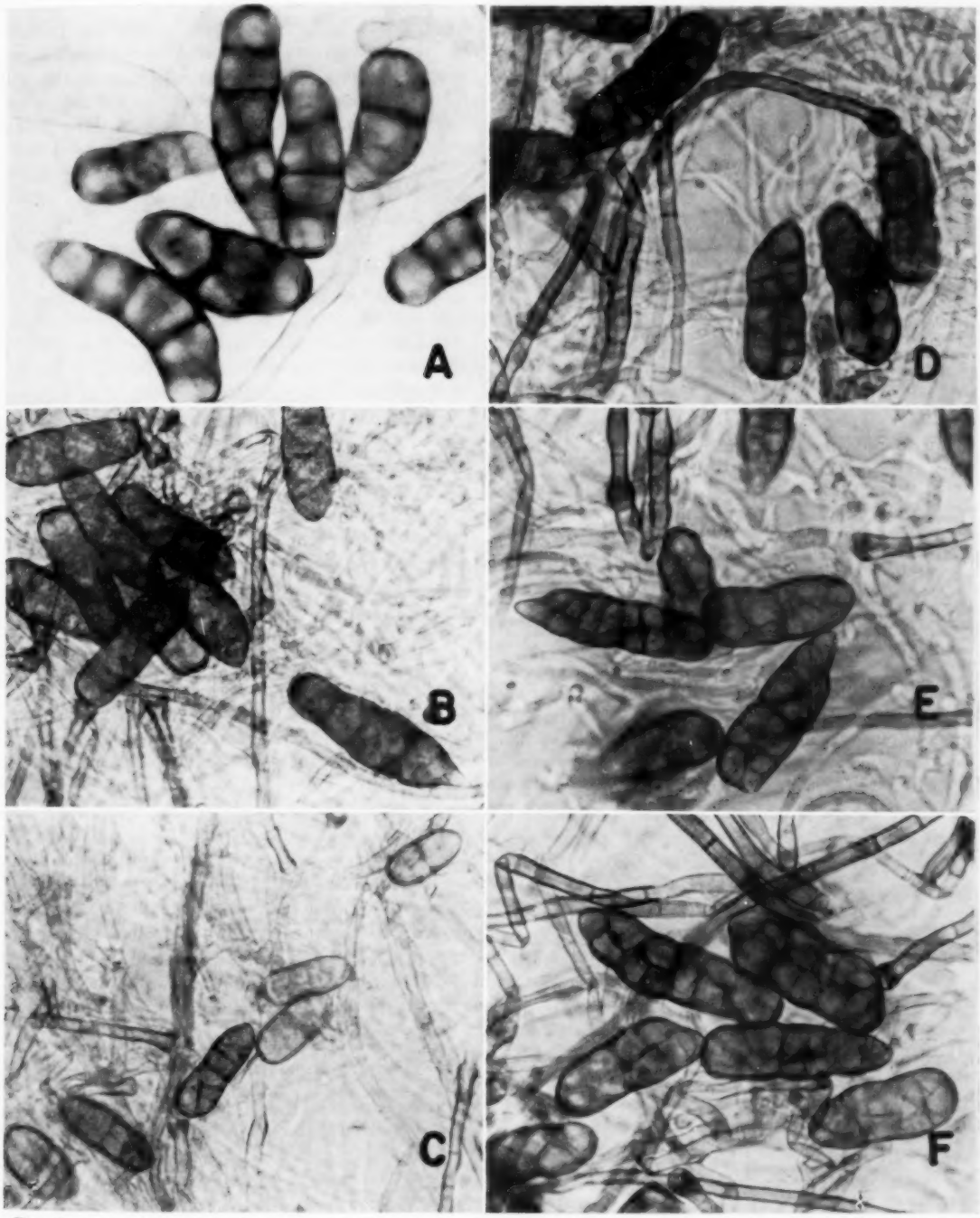


**Fig. 2.**—Conidia of leguminicolous and related species of *Stemphylium* grown on V8-juice agar. **A)** *S. callistephi* from China aster; **B)** *S. floridanum* from tomato; **C)** *S. trifolii* from Ladino white clover; **D)** *S. solani* from tomato; **E)** *S. solani* from blue lupine; **F)** *S.* sp. from crown vetch. (×500).

*esculentum* Mill.) and 4 from blue lupine; 7) 3 isolates of *S. trifolii* Graham from Ladino white clover (2 states); 8) one isolate of *S. floridanum* Hannon & G. F. Weber from tomato; and 9) one isolate of *S. callistephi* Baker & Davis from China aster (*Callistephus chinensis* (L.) Nees).

Each isolate was tested on the following seedlings: alfalfa, red clover, Ladino white clover, birdsfoot trefoil, blue lupine, crown vetch, and tomato. In addition, *S. sarcinaeforme* was tested on white sweet clover. About 10 seedlings, 6–8 weeks old, in each of 3 pots were inoculated with mycelial and conidial suspensions of each isolate. Inoculated seedlings were then covered with transparent polyethylene bags, protected from direct sunlight for 2 days, and kept at 20–25°C on a greenhouse bench.

Spore size, morphology, and cultural characteristics of 20 *Stemphylium* isolates were also recorded from 7-day-old cultures grown on V8-juice agar.

**Pathogenicity.**—Isolates of *S. botryosum* differed greatly in host range. Isolates from alfalfa and red clover were pathogenic to the aboveground parts of 5 of the 7 plant species, whereas those from blue lupine and tall fescue were nonpathogenic. Infection was greatest on alfalfa, and only light on red clover, Ladino white clover, blue lupine, and crown vetch. The small-spored *Stemphylium* isolates from Ladino white clover and crown vetch were not pathogenic to any of the test plants. Isolates of *S. sarcinaeforme* from red clover, Ladino white clover, and white sweet clover readily infected these hosts but were less infectious to alfalfa. Three isolates of *S. loti* from birdsfoot trefoil were equally pathogenic to birdsfoot trefoil and blue lupine. *S. trifolii* was most pathogenic to Ladino white clover, but attacked red clover and alfalfa mildly.

*S. floridanum* and *S. solani* from tomato infected only tomato. The *Stemphylium* from blue lupine reported by Wells to be *S. solani* caused severe defoliation of blue lupine but was mildly pathogenic to tomato. Isolates from crown vetch, morphologically somewhat similar to *S. solani*, infected tomato and crown vetch slightly. *S. callistephi* was not pathogenic on any of the plants used in the experiment.

**Morphology.**—According to the concept of the genus *Stemphylium* proposed by Wiltshire (4), all the isolates studied would be classified in the *Eustemphylium* group. Mature conidia of all isolates were constricted at the median septum, and those of *S. trifolii*, *S. floridanum*, and *S. callistephi* usually had 1 or more additional constrictions. Conidia of the last 3 species, *S. solani*, and the large-spored isolates from crown vetch were tapered toward the apex, whereas conidia of *S. botryosum*, *S. sarcinaeforme*, *S. loti*, and the small-spored isolates from Ladino white clover and crown vetch were sarciniform.

Isolates were distinguished further on the basis of conidial size, smoothness of outer spore wall, and production of perithecial or sclerotial bodies (Table 1). *S. botryosum*, *S. sarcinaeforme*, and *S. loti* were readily differentiated on these bases. Conidia of the unidentified isolates from Ladino white clover were larger than conidia of the small-spored isolates from crown vetch. Isolates of both had conidia with slightly rough walls (Fig. 1-D,E,F). Isolates from Ladino white clover produced sclerotium-like bodies in the medium, whereas isolates from crown vetch produced perithecia containing ascospores similar to those of *Pleospora herbarum* Rab.

The isolates differed culturally although conidia of the large-spored isolates from crown vetch resembled those of *S. solani* from tomato and lupine in size, shape, and smoothness of outer wall. Colonies of *S. solani* from lupine and tomato were light to dark gray and generally zonate, whereas colonies of crown vetch isolates were gun-metal gray to mouse gray and not zonate. Conidia of *S. floridanum* had rough walls and more constrictions than conidia of *S. solani* (Fig. 2-B,D,E). Conidia of *S. callistephi* appeared to be slightly rough-walled and often bent or curved.

**Discussion.**—The results of pathological and morphological studies suggest that the unidentified isolates from Ladino white clover and crown vetch differ from each other and from other leguminicolous species of *Stemphylium*. The most pronounced differences were in pathogenicity, the unidentified small-spored isolates from crown vetch and Ladino white clover being nonpathogenic to the plants tested. The large-spored isolates from crown vetch were slightly pathogenic to crown vetch and tomato. Since these isolates from crown vetch were somewhat similar morphologically but different pathogenically from *S. solani* on tomato and blue lupine, the differences may be attributed to pathogenic specialization. Isolates of *S. botryosum* from various hosts also differed in pathogenicity.

*Stemphylium botryosum* has long been known to attack a wide range of hosts, but this is the first report of its being pathogenic on crown vetch. Neergaard (2) reported tomato to be susceptible to *S. botryosum*; in the present studies tomato plants were not attacked. Susceptibility of blue lupine to *S. loti* is also reported in the present paper.

It is noteworthy that none of the large-spored *Stemphylium* isolates was pathogenic to the forage legumes tested. The small-spored isolates from crown vetch produced an ascigerous stage resembling *Pleospora herbarum* and conidia with slightly rough walls, suggesting that this fungus is closely related to *S. botryosum*.—U. S. Regional Pasture Research Laboratory, University Park, Pennsylvania.

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# INFLUENCE OF ENVIRONMENT ON DISEASES OF TURFGRASSES. II. EFFECT OF NUTRITION, pH, AND SOIL MOISTURE ON SCLEROTINIA DOLLAR SPOT

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Contribution No. 257, Department of Botany and Plant Pathology, Pennsylvania Agricultural Experiment Station. Authorized for publication February 3, 1960, as paper No. 2435 in the Journal Series.

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SUMMARY

Kentucky bluegrass (*Poa pratensis*) was grown in 8 in. glazed crocks, with silica sand as the supporting medium. Various levels of nutrition were supplied with a continuous-flow drip-culture system. Post-emergence inoculation with *Sclerotinia homoeocarpa* was performed by adding aqueous mycelial suspensions around the crowns of the plants. Under low nitrogen nutrition, with normal levels of phosphorous and potassium, or under low balanced nutrition, disease severity decreased significantly. Neither high nor low levels of phosphorous and potassium, high nitrogen, nor high balanced nutrition influenced disease development. Altering hydrogen-ion activity from pH 4.0 to pH 9.0, at different nutritional levels, did not affect disease proneness. The influence of 5 soil moisture levels in the readily available range on disease development was studied by varying frequency of irrigations prior to inoculation. Disease development was significantly greater with irrigation practices that produced higher soil moisture stresses, i.e., allowing the plants to extract to $^3/_4$ field capacity and below.

Sclerotinia dollar spot is one of the more common fungus foliar diseases of turfgrasses. The incitant, *Sclerotinia homoeocarpa* F. T. Bennett (1), attacks a wide range of grass species, including members of the genera *Poa*, *Festuca*, and *Agrostis* (4). Generally recognized as a disease problem of golf course putting greens, Sclerotinia dollar spot may also be severe on home lawns, park lawn areas, and golf course fairways. In the latter cases, the importance of the disease is not fully realized since symptomatology varies with management practices. With these conditions of higher cutting, and usually few, if any, fungicide applications, the rather small, sharply outlined spots of infection that occur on the putting green are replaced by large, irregularly shaped areas of blighted turfgrass, measuring up to 12 feet in diameter. In this form, it is not uncommon for the disease to be erroneously diagnosed as Curvularia blight, nematode damage, drought injury, fertilizer damage, etc.

Concerning nutrition and soil moisture and the development of Sclerotinia dollar spot, Monteith and Dahl (5) observed, "The disease is discouraged by the judicious use of fertilizer and water." Howard, Rowell, and Keil (4) reported the disease to be more noticeable under conditions of high soil moisture stress. That high nitrogen fertility is conducive to less disease development has been attested to by Runnels (7), Younger (9), and Smith (8). Davis (3) observed that Sclerotinia dollar spot is usually more severe on insufficiently fertilized lawns, and Musser (6) reported, "Either starvation or an excess of nitrogen will intensify dollar spot."

In the present investigation, the influence of nutrition and pH on the development of *S. homoeocarpa* on Kentucky Bluegrass (*Poa pratensis* L.) was studied in a continuous-flow drip-culture system, with silica sand as the supporting medium (2).

Each crock, seeded to contain about 200 plants, was irrigated periodically with distilled water until the seeds germinated and the plants were established. Then, modified Hoagland's solutions were added to the crocks by means of the drip-culture system. As in a previous investigation (2), the basal solution (1.0 H) was modified to 0.1 H and 3.0 H concentrations. In addition, the 1.0 H formula was modified to produce nutritional levels of 0.1× (low) and 3.0× (high) concentrations of nitrogen, phosphorous, and potassium. All solutions were adjusted to pH 5.6 with NaOH or HCl as required. Minor element concentration was the same in all the solutions. In the nutritional series, then, the various modifications of the basal solution produced 9 different combinations. These were all run simultaneously through 5 replications of 4 crocks each. All experiments were conducted at 70°F.

The effect of pH on disease development was studied at low nitrogen, high nitrogen, and 1.0 H nutritional levels. The 2 nitrogen series were adjusted to pH 4.0, 5.6, 7.0, and 9.0, whereas the 1.0 H level was adjusted to pH 5.6 and 9.0. The pH was altered with NaOH or HCl as required.

The pH and nutritional studies were conducted concurrently. Inoculation was accomplished when the plants were 12 weeks old.

Inoculum for the nutrition and pH series was prepared by growing *S. homoeocarpa* on malt-extract agar in Petri plates until mycelial growth completely covered the agar surface. The contents of 300 plates were then chopped in a Waring blender and the resulting agar-mycelium mixture was brought to 20 L with distilled water. After 100 ml of the mycelial suspension was poured around the plants, each crock was covered with a moistened plastic bag. Five days later the plastic bags were removed, and after 1 week more, disease readings were made. The disease readings were based on an actual count of individual plants per crock, and incidence was recorded as per cent dead plants per treatment. All data were subjected to analysis of variance.

Disease incidence was significantly less at low nitrogen or 0.1 H than at 1.0 H. Neither high nitrogen, 3.0 H nutrition, nor phosphorous and potassium vari-

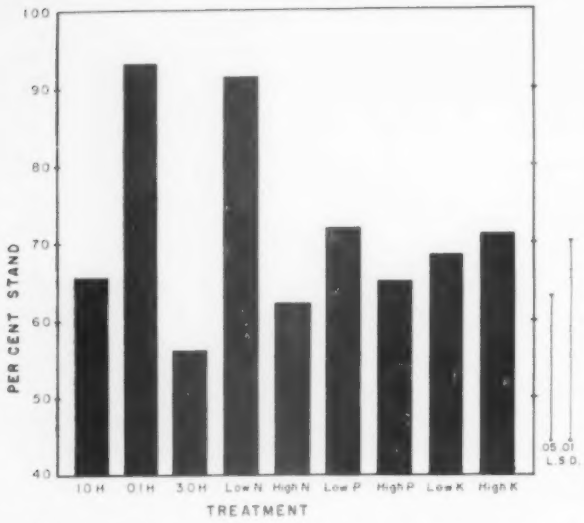


**Fig. 1.**—Influence of nutrition on the susceptibility of Kentucky bluegrass to *Sclerotinia homoeocarpa.*

ables influenced disease development (Fig. 1). The various pH levels did not alter the disease response characteristic of the nutritional levels studied.

The influence on Sclerotinia dollar spot of soil moisture in the readily available range—field capacity (FC) to permanent wilting percentage (PWP)—was studied by varying irrigation frequency (2). Using Hagerstown silt loam, with a moisture equivalent of 24.5% and a PWP of 10.1%, 100 non-porous containers were filled with a standard weight of soil of known moisture content. The containers were then seeded to Kentucky bluegrass, and all were maintained at FC, by weight, until the roots had permeated the soil masses. With the completion of root permeation, the following soil moisture stress groups, comprising 20 containers each, were established: FC, ¾ FC, ½ FC, ¼ FC, PWP. For a 2-week period, the plants were allowed to extract moisture to the predetermined soil moisture stress, as established by the total weight of the system, and then irrigated back to the FC weight.

Inoculum for the soil moisture series was prepared by growing *S. homoeocarpa* on an autoclaved rye grain medium, air-drying the fungus-permeated grains, and then grinding them in a food mill. Inoculation was accomplished by first allowing the plants of each container to extract moisture, by weight, to the predetermined stress point. At this time, a standard amount of the finely ground grain inoculum was scattered around the base of the plants, and the respective containers were covered with moistened plastic bags. Five days later, the plastic bags were removed, and after 1 week more, disease readings were made. Disease comparisons were made on a severity scale of 0–10, from no leaf blighting to 100% foliar loss. All data were subjected to analysis of variance.

Kentucky bluegrass plants grown at high soil moisture stress, i.e., extracting to ¾ FC and below, were more susceptible to *S. homoeocarpa* than those produced at continuous FC (Fig. 2). This difference in severity was very distinct in the ¼ FC and PWP extraction series, in which foliar blighting was more than double that of plants grown at FC.

**Discussion.**—The results do not support the general consensus that turfgrass grown at low nitrogen fertility is more prone to Sclerotinia dollar spot. To the contrary, Kentucky bluegrass, at least, is less susceptible to *S. homoeocarpa* under low nitrogen nutrition than under normal or high nitrogen fertility.

Since the majority of reports to date have been based primarily on field observations, it is suggested that the reported beneficial effect of high nitrogen fertility could have been one of more rapid plant recovery during periods of lessened activity on the part of the parasite, rather than a legitimate resistance to infection.

The role of soil moisture in Sclerotinia dollar spot development is quite pronounced and could easily complicate field studies concerned with fertility and disease incidence. The heightened disease proneness of Kentucky bluegrass at high soil moisture stress presents a possible explanation of the sometimes epiphytotic occurrence of this particular disease in seasons of low rainfall.

An interesting aspect of the role of soil moisture and the susceptibility of Kentucky bluegrass to *S. homoeocarpa* is that this facet of the environment can alter the level of severity of a foliar disease so appreciably. This illustrates further the potential of soil moisture as a factor in influencing the magnitude

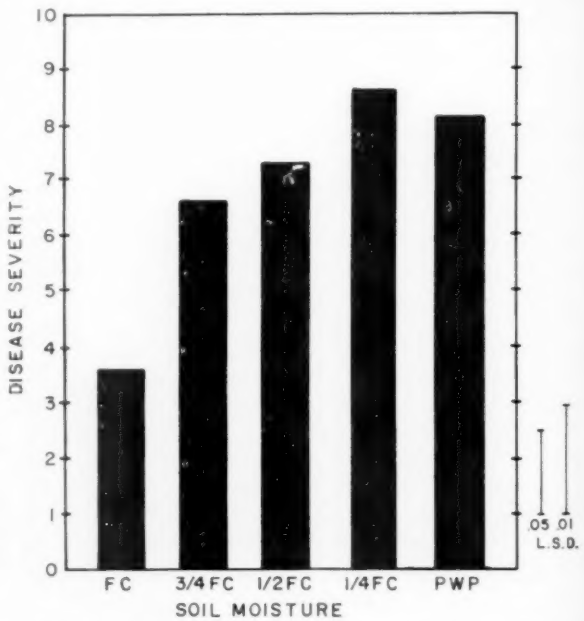


**Fig. 2.**—Effect of soil moisture stresses in the readily available range on the susceptibility of Kentucky bluegrass to *Sclerotinia homoeocarpa.* 0 = no leaf blighting; 10 = 100% foliar loss.

of disease development. Its regulation, therefore, through standardization of stresses within an experiment, and from one experiment to the next, is extremely important in highly critical investigations—whether concerned with root, crown, or foliar diseases. —Department of Botany and Plant Pathology, The Pennsylvania State University, University Park.

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# ETIOLOGY OF WILT IN FUSARIUM-INFECTED COTTON

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SUMMARY

Cotton plants inoculated with 23 species of *Fusarium* fluoresced under ultraviolet light only when colonized by *Fusarium vasinfectum*. Likewise, *F. vasinfectum* neither infected nor incited fluorescence in crop plants other than cotton grown in soil infested by the fungus. In wilt-infected cotton plants fluorescence appeared in the endodermis of the roots on the fifth day after germination in the infested soil and spread progressively into medullary rays, phloem, and xylem. Fluorescence spread acropetally as the fungus invaded the plant-axis during subsequent days of pathogenesis. Cotyledonary and adult leaves and their petioles fluoresced in spite of the absence of the pathogen in them. Fusaric acid, though not responsible for the fluorescence, was detected in plants even on the second day after germination in the infested soil and reached a maximum on the 13th day.

**Introduction.**—In an earlier report (6) the author indicated that cotton plants infected with *Fusarium vasinfectum* Atk. fluoresced in ultraviolet light in the dark, and that this was useful as a diagnostic measure for early evaluation of the disease. Herein are further findings on the etiology of wilt in cotton plants infected by *F. vasinfectum*.

**Materials and methods.**—The cultures of *Fusaria* used, from Centraalbureau voor Schimmelcultures, Holland, were: *F. lateritium* Nees var. *uncinatum* Wr., *F. sambucinum* Fekl., *F. culmorum* (W. Sm.) Sacc., *F. orthoceras* App. & Wr., *F. oxysporum* Schlecht., *F. conglutinans* Wr., *F. oxysporum* Schlecht. f. *nicotianae* (Johnson) Snyd. & Hans., *F. lini* Bolley, *F. equiseti* (Cda.) Sacc., *F. poae* (Pk.) Wr., *F. sporotrichioides* Sherb., *F. buharicum* Jacz., *F. solani* (Mart.) App. & Wr., *F. caucasicum* Let., *F. bulbigenum* Cke. & Mass. var. *lycopersici* (Brushi) Wr. & Rkg., *F. dimerum* Penz., *F. chlamydosporum* Wr. & Rkg., *F. javanicum* Koord, *F. scirpi* Lamb. & Fautr., *F. semitectum* Atk., and *F. vasinfectum* Atk. In addition to these, *F. udum* Butl. was isolated from wilt-infected red-gram plants (*Cajanus cajan* Adans.), and *F. moniliforme* Sheld. was obtained from the Madras Agricultural College, Coimbatore. Air-dried and sieved garden soil in earthenware pots sterilized for 2 hours at 20 lb steam pressure was used in the infection studies. Cultures growing in sterilized soil-oats-water mixture (9:1:3) were used as inocula. The fungus thus grown was thoroughly mixed and transferred to sterilized soil at the rate of 5 g of inoculum to every 100 g of soil. The inoculum and the soil were mixed well and incubated for 48 hours on a greenhouse bench at a temperature of 25–35°C. The initial moisture level was adjusted to 50%, and seeds of a variety of cotton susceptible to *F. vasinfectum* wilt, Karunganni 2, *Gossypium arboreum* Linn. race *indicum*, were sown. After germination, enough water was added to prevent wilting. Philips H.P.W. 125-W uv lamp, 3655Å, was used to detect the infected plants.

**Table 1.**—Percentage of transverse sections of cotton plants infected with *Fusarium vasinfectum* showing fluorescence along the axis.

| Plant age | In. belowground | | In. aboveground | | | Cotyledonary leaves | |
|---|---|---|---|---|---|---|---|
| (days) | 2 | 1 | 1 | 2 | 3 | Petioles | Laminae |
| 1–2 | 0 | 0 | 0 | – | – | 0 | 0 |
| 3–5 | 0 | 0 | 0 | 0 | – | 0 | 0 |
| 6 | 25 | 15 | 0 | 0 | 0 | 0 | 0 |
| 7 | 15 | 10 | 0 | 0 | 0 | 0 | 0 |
| 8 | 55 | 45 | 0 | 0 | 0 | 0 | 0 |
| 9 | 40 | 35 | 0 | 0 | 0 | 0 | 0 |
| 10 | 65 | 55 | 45 | 35 | 0 | 0 | 0 |
| 11 | 75 | 45 | 20 | 15 | 10 | 10 | 10 |
| 12 | 55 | 50 | 25 | 25 | 20 | 15 | 15 |
| 13 | 45 | 45 | 40 | 35 | 30 | 25 | 25 |
| 14 | 65 | 60 | 50 | 40 | 30 | 20 | 20 |

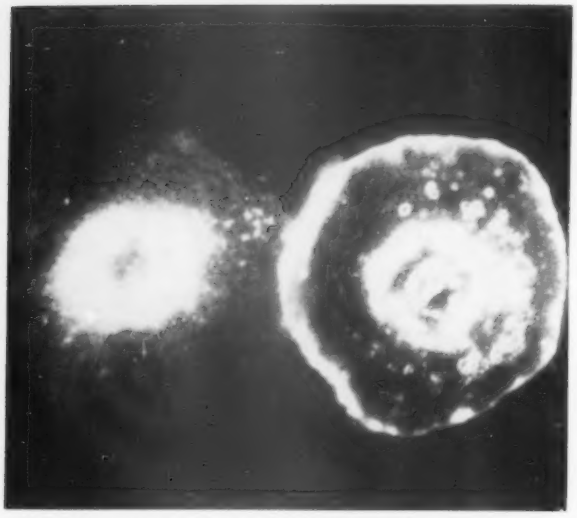

**Fig. 1.**—Photomicrograph of free-hand transverse section of root and stem of a 14-day-old wilting and necrosed cotton plant under ultraviolet light.

**Results.**—*Invasion of the pathogen and progress of fluorescence.*—Free-hand transverse sections from different heights along the plant axis, including the cotyledonary leaves and the petioles, of 20 plants chosen at random were examined microscopically under uv light in dark each day for 14 days from germination in the infested soil. The results are in Table 1. Fluorescence was usually manifested in the isolated cells of the endodermis on the 6th day, and then gradually progressed to medullary rays, phloem, and xylem. Transections of root and stem of necrosed and flaccid plants, however, showed bright fluorescence of the entire vascular bundle (Fig. 1). External observations indicated an acropetal progression of fluorescence of stem from the 10th day, culminating in petiolar and veinal fluorescence. Simultaneous isolation of the fungus from infected plant parts, including the axis, cotyledonary leaves, and petioles, over a similar period showed that *F. vasinfectum* could be recovered from the roots on the second day. The results (Table 2) also indicated a gradual invasion of the pathogen throughout the plant-axis, except in the petioles and the cotyledonary leaf-laminae. A similar plating-out of plant parts from different heights of the plant-axis of 20 cotton plants infected for 2 months and displaying fluorescent axes, petioles, and veins revealed that the fungus was recoverable to the very apex of the axis. The petioles of the cotyledonary leaves never yielded the pathogen, and the leaf-laminae of both the adult and cotyledonary leaves were likewise free of the fungus.

To find out to what extent fluorescence was a specific expression of cotton plants infected by *F. vasinfectum* they were inoculated with the 23 species of *Fusaria* mentioned earlier and examined under uv light once a day for 4 weeks. Only cotton plants infested with *F. vasinfectum* fluoresced and yielded the fungus when roots were plated-out.

To determine whether *F. vasinfectum* is capable of inducing fluorescence in plants other than cotton, six crop plants, *Eleusine coracana* Gaertn., *Oryza sativa* Linn., *Cajanus cajan* Adans, *Lycopersicum esculentum* Mill., *Arachis hypogea* Linn. and *Capsicum frutescens* Linn., were grown in sterilized soil inoculated with *F. vasinfectum*, and observed for 8 weeks. None of these plants fluoresced or yielded the fungus when their roots were plated-out. Within the limits of the experiment, this fluorescence of plant parts seems specific to cotton infected by *F. vasinfectum*.

*Daily variations in the in vivo fusaric acid in infected plants.*—Fusaric acid has been demonstrated in cotton plants infected by *F. vasinfectum* (3, 4, 7). Therefore, the fusaric acid content of infected plants during a 14-day period of pathogenesis was studied. Disease intensity as revealed under uv light was scored as follows: 1 = plants showing fluorescence of stem alone, 2 = plants showing fluorescence of stem and in addition showing vein-clearing visible to the unaided eye (5), 3 = plants brightly fluorescent and necrosed. The number of plants in each grade was multiplied by its score, the sum of the products again multiplied by 100 and divided by 3 times the total number of plants in each replicate. The mean of the replicates served as the wilt-index denoting the severity of the disease. Wilt index was recorded daily for 14 days. On each day, 10 g (fresh weight) of infected whole plants were washed in glass-distilled water and ground with acid-washed river sand and 10 ml distilled water. The extract was centrifuged, pH of the supernatant noted (colorimetric), and the supernatant concentrated to 2 ml over sulphuric acid in a desiccator under vacuum at 4°C. This concentrated extract was used to assay fusaric acid by the agar-cup technique (2) based on the capacity of the toxin to inhibit the growth of a selected soil bacterium. Variation in the *in vivo* fusaric acid content, progression in wilt, and changes in pH of the sap for 14 days following germination are presented in Table 3.

**Table 2.**—Percentage recovery of *Fusarium vasinfectum* from parts along the axis of infected cotton plants.[a]

| Plant age | In. belowground | | In. aboveground | | |
|---|---|---|---|---|---|
| (days) | 2 | 1 | 1 | 2 | 3 |
| 1 | 0 | 0 | 0 | – | – |
| 2 | 45 | 35 | 0 | – | – |
| 3 | 25 | 25 | 0 | 0 | – |
| 4 | 35 | 30 | 0 | 0 | – |
| 5 | 35 | 30 | 5 | 0 | – |
| 6 | 45 | 15 | 15 | 0 | 0 |
| 7 | 45 | 10 | 5 | 0 | 0 |
| 8 | 65 | 55 | 75 | 25 | 0 |
| 9 | 45 | 55 | 25 | 20 | 0 |
| 10 | 80 | 60 | 60 | 60 | 20 |
| 11 | 85 | 50 | 35 | 30 | 30 |
| 12 | 60 | 55 | 30 | 30 | 20 |
| 13 | 60 | 60 | 35 | 35 | 20 |
| 14 | 70 | 65 | 40 | 30 | 30 |

[a] Petioles of cotyledonary leaves and the leaf-laminae did not yield the pathogen at any age level studied.

**Table 3.**—Relation of age of cotton plants infected with *Fusarium vasinfectum* to pH of sap, disease intensity, and fusaric acid content.

| Plant age (days) | Fusaric acid[a] | pH | Wilt-index[b] |
|---|---|---|---|
| 1 | 0.0 | 6.4 | 0.0 |
| 2 | 1.0 | 6.2 | 0.0 |
| 3 | 0.0 | 6.0 | 0.0 |
| 4 | 0.0 | 5.6 | 0.0 |
| 5 | 0.0 | 5.6 | 0.0 |
| 6 | 0.0 | 7.2 | 5.6 |
| 7 | 0.0 | 7.2 | 5.6 |
| 8 | 0.0 | 7.3 | 5.6 |
| 9 | 0.0 | 7.2 | 8.8 |
| 10 | 0.0 | 7.0 | 8.8 |
| 11 | 0.0 | 7.4 | 16.6 |
| 12 | 1.12 | 7.2 | 35.5 |
| 13 | 1.52 | 7.2 | 42.6 |
| 14 | 0.96 | 7.4 | 50.4 |

[a] Milligrams of acid per 100 g fresh weight.

[b] 1 = stem fluorescing, 2 = stem fluorescence + vein clearing, 3 = brightly fluorescent and necrosed plants. Number of plants in each grade multiplied by its score, and the sum of the products multiplied by 100 and divided by 3 times the total number of plants.

Though fusaric acid was detected even on the second day, it was not detectable again until the 12th day. On the 12th, 13th, and 14th days the toxin was once again detected, being maximum on the 13th day, when wilt-index also was high. The pH of the sap did not alter radically during infection.

**Discussion.**—Comparison of results in Tables 1 and 2 reveals that invasion of the cotton plant by *F. vasinfectum* and fluorescence progress in a parallel manner except for the absence of fluorescence during the first 5 days, even though the roots were colonized by the fungus from the 2nd day. This suggests the necessity of an initial period of interaction between the pathogen and the host as a prerequisite for fluorescence. According to Gäumann (1), toxicity of fusaric acid to tomato stems at a dosage of 150 mg per kg fresh weight in a concentration of $2.5\times10^{-3}M$ was zero at pH 6.2, whereas the toxicity rating for leaves at the same pH and dosage was 1.37. This difference in fusaric acid activity in relation to pH and the nature of tissue on which it acts has been attributed to the high degree of its dissociation at reactions above pH 6.0 (1). On the 2nd day of infection, the sap of infected cotton plants had a pH of 6.2 and contained 1 mg fusaric acid per 100 g fresh weight of plant tissue (Table 3). No data are available on the influence of pH of fusaric acid on its toxicity to cotton plants. Therefore, assuming that fusaric acid in cotton plants behaves in a similar manner as it does in tomato plants, the following argument could be advanced to explain the fate of the toxin detected on the 2nd day. In its highly dissociated state at pH around 6.0, the migration of fusaric acid molecules among cells through the plasma membrane has been hypothesized to be at a minimum, thus resulting in a very low utilization of the toxin by tomato stems (1). It is possible to visualize similar events in 2-day-old infected cotton plants, leading to the decreased activity of fusaric acid. Although at pH 6.0 greater damage to leaf tissue was possible, the quantity of the toxin left over after utilization by the stem could have been so little that it hardly reached the threshold necessary for foliar injury. Besides, the concentration of fusaric acid in the plant was so little, and further, the interplay of several unknown factors involved in host-parasite relationship of cotton wilt has to be taken into account in any attempt to explain the mechanism of fusaric acid activity *in vivo*.—University Botany Laboratory, Madras 5, India.

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# DOMINANCE OF AVIRULENCE AND MONOGENIC CONTROL OF VIRULENCE IN RACE HYBRIDS OF USTILAGO AVENAE

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SUMMARY

The mode of inheritance of pathogenicity was investigated in 9 interracial hybrids involving 7 races of *Ustilago avenae*. Avirulence was dominant in all 9 hybrids. Dominance was complete in the majority of cases and almost complete in the others. Virulence was inherited in monogenic $F_2$ ratios of 3A to 1V. In hybrids involving a multiplicity of virulence genes, segregation and recombination gave rise to new virulence types (races).

**Introduction.**—Pathogenic differences among subspecies entities of *Ustilago avenae* (Pers.) Rostr. reflect genetic phenomena of basic significance to the identity and integrity of specialized races of this species, particularly with respect to establishing and maintaining systems of race classification. Several workers (5, 6, 8) have encountered unstable pathogenic patterns in certain race categories. Also pertinent to this problem, Cherewick (1) emphasized the role of persistent heterozygosity for pathogenicity in the races of several smut species, including *U. avenae*. Repeated passage through the host tester varieties produced pathogenic homozygosity in some races whereas others remained heterozygous after as many as 10 passages. Even some of the monosporidial line combinations that might have been expected to be homozygous were among the pathogenically unstable lines (1).

Nicolaisen (7) had previously shown that individual spores of *U. avenae* might be heterozygous for pathogenicity and that, in a single hybrid, virulence and avirulence might be either dominant or recessive, depending on the host variety. Halisky (2) likewise concluded that avirulence was dominant in certain race hybrids of *U. avenae* and recessive in others, but his interpretation is subject to some revision (4). Thus the problem of instability of pathogenicity patterns in races of *U. avenae* involves not only heterozygosity but also the number of specific factors for pathogenicity and their mode of inheritance, which in turn poses the question of whether virulence or avirulence is dominant in race hybrids. The results of studies to clarify these points in certain race hybrids are presented here.

**Materials and methods.**—Four standard differential varieties of oats and 7 races of *U. avenae* (5) were used. The inoculations were made by vacuum pump with inoculum composed of paired compatible monosporidial lines isolated by standard methods (2). Compatible reactions between monosporidial lines were determined by mixing sporidia of two lines on agar and identifying compatible pairs from sporidial fusions observed with a microscope.

Pathogenicity was measured in terms of infection percentages, which were based on total panicles to the row in the field and total plants to the row in the greenhouse. The only differential pattern measured was virulent vs. avirulent, for which the ideal respective infection levels are 100 and 0. Although only a few 100% infections occurred, the great majority of the virulent-reaction designations were based on infections ranging from 50% (predominantly above 70), with only a few below the 50% level. In contrast, more than three-fourths of the avirulent designations were based on 0 infections, and the remainder were based on relatively low infections, which ranged up to 26% (predominantly below 10).

**Results.**—*Dominance of avirulence.*—The reactions of 7 races of *U. avenae* and 9 hybrids on 4 differential varieties of oats are shown in Table 1, along with a theoretical genetic analysis of pathogenicity in each race. The analysis indicated is based on a single recessive gene for each virulent pattern. The virulent and avirulent patterns indicated represent average infections produced by 4 or more pairs of monosporidial lines of each race and 8 or more pairs of hybrid combinations representing each of the various race crosses. The differential pattern between races and the comparative or contrasting pattern of related hybrid combinations are shown by virulent (V) and avirulent (A) patterns on one or two of the host varieties (Table 1).

Dominance of avirulence is evident in every hybrid combination shown (Table 1). Thus, the $F_1$ pattern of the various race hybrids resembled one parent in some instances and neither parent in others. For example, the hybrid between races 1 and 2, which differ only in their respective avirulent and virulent patterns on Camas, displayed a typical race 1 host range. In contrast, the hybrid between races 2 and 3 resembled neither parent in host range. In this hybrid the avirulence of race 2 on the variety Monarch and of race 3 on Camas was dominant over the virulence of races 3 and 2, respectively, on these same varieties. Hence, in the $F_1$, hybrid 2×3, like 1×2, displayed the host range of race 1. Hybrids 2×7 and 2×7b exhibited the A-2 host range, reflecting dominance of race 2 avirulence on Gothland and Monarch.

The reactions indicated for hybrids of races 5×6 and 5×7 also reflect dominance of avirulence and are based on data reported by Halisky (2). The hybrid $F_1$ dicaryons were found to be only weakly pathogenic (1–9% infection), suggesting incomplete dominance of virulence. A revision of this interpretation followed, as discussed by Holton (4), and it is now conceded

**Table 1.**—Patterns of pathogenicity and theoretical genotypes of 7 races and 9 interracial hybrids of *Ustilago avenae* on four differential varieties of oats.

| *U. avenae* | Oat variety and race reaction[a] | | | | Genetic analysis[b] | |
|---|---|---|---|---|---|---|
| | Anthony | Gothland | Monarch | Camas | Genotype | Phenotype |
| Race: | | | | | | |
| 1 | V | A | A | A | aaGGMMCC | A-1 |
| 2 | V | A | A | V | aaGGMMcc | A-2 |
| 3 | V | A | V | A | aaGGmmCC | A-3 |
| 5 | V | V | A | A | aaggMMCC | A-5 |
| 6 | V | V | V | A | aaggmmCC | A-6 |
| 7 | V | V | V | V | aaggmmcc | A-7 |
| 7b | V | V | A | V | aaggMMcc | A-7b |
| Cross: | | | | | | |
| 1×2 | V | A | A | A | aaGGMMCc | A-1 |
| 2×3 | V | A | A | A | aaGGMmCc | A-1 |
| 2×7 | V | A | A | V | aaGgMmcc | A-2 |
| 2×7b | V | A | A | V | aaGgMMcc | A-2 |
| 5×6 | V | V | A | A | aaggMmCC | A-5 |
| 5×7 | V | V | A | A | aaggMmCc | A-5 |
| 6×7b | V | V | A | A | aaggMmCc | A-5 |
| 6×7 | V | V | V | A | aaggmmCc | A-6 |
| 7×7b | V | V | A | V | aaggMmcc | A-7b |

[a] V = virulent; A = avirulent phenotypes.
[b] Virulence on Anthony, Gothland, Monarch, and Camas is designated by symbols aa, gg, mm, and cc, respectively.

that low infections by $F_1$ hybrid lines, like 0 infections, also indicate avirulence and that avirulence is dominant in hybrids 5×6 and 5×7. Hybrid dicaryons representing the cross 6×7, on the other hand, show complete dominance of avirulence (2, 3). The A-5 pattern displayed by hybrid 6×7b reflects the avirulence of race 6 on Camas and of A-7b on Monarch. The avirulence of A-7b on Monarch was also expressed by the hybrid of 7×7b (Table 1).

The genetic analysis indicated in Table 1, for the individual races and hybrids between them, is based on the evidence that a single gene conditions the differential virulence of two races on the same host variety (Tables 2, 3, 4). On the premise that avirulence is dominant, and expanding Halisky's (2) system of gene symbols (exclusive of complementary genes), *aa*, *gg*, *mm*, and *cc*, respectively, denote virulence on Anthony, Gothland, Monarch, and Camas. Thus, the genotype of each race is homozygous for virulence and avirulence as exemplified by its pathogenic pattern on the four host varieties (Table 1). For example, the host range of race 1 reflects the genotype aaGGMMCC, denoting virulence (aa) on Anthony only, whereas the race 7 genotype (aaggmmcc) denotes virulence on all four varieties. Such an analysis assumes that the gene for virulence on Anthony is also present and recessive (aa) in all 7 races, a fact not demonstrable without differential reaction on Anthony.

The various combinations of the genes indicated for races 1 and 7 in homozygous condition account for the pathogenicity patterns (phenotypes) of the other 5 races (Table 1). Extension of this analysis to the hybrids between races emphasizes the significance of the genotype-phenotype relationship to the identity and stability of pathogenic races. Thus, phenotypically, hybrids 1×2 and 2×3 display the host range of race 1, thereby reflecting directly the dominance of avirulence. Genotypically, however, both are heterozygous, 1×2 for virulence on Camas and 2×3 for virulence on the varieties Monarch and Camas. Hybrids 5×6 and 5×7 are phenotypes of race 5, although heterozygous, respectively, for virulence on Monarch, and on Monarch and Camas. Likewise, hybrid 6×7b is a phenotype of race 5, but genotypically is heterozygous for virulence on Monarch and Camas. The remainder of the hybrids included in Table 1 (2×7, 2×7b, 6×7, 7×7b) involve gene differences that combine into phenotypes resembling in each case the parent race that has the lower number of recessive genes for virulence. All of these $F_1$ hybrids, therefore, are heterozygous for virulence on their respective differential varieties.

The foregoing analysis has self-evident implications with respect to establishing and maintaining genetically stable pathogenic races. It is further evident that host screening, as an approach to homozygosity, is greatly enhanced by dominance of avirulence, inasmuch as strong virulence on a differential variety connotes homozygosity for that reaction. On the contrary, should virulence be dominant (7) the kind of variability in pathogenic patterns that has been reported (1, 5, 6, 8) can be expected.

*Monogenic control of virulence.*—Further information on the mode of inheritance and the number of factors governing virulence in *Ustilago avenae* may be obtained by selfing $F_1$ race hybrids. Each selfed $F_1$ spore gives rise to 4 dicaryons constituting an $F_2$ population segregating for avirulence and virulence. Since the original crosses (Table 1) represent in each instance "avirulence × virulence," the resulting $F_1$ hybrids are heterozygous with avirulence expressed as the dominant character. The ratio of avirulent to

**Table 2.**—The infection percentages, accompanied by relevant gene symbols, produced on Gothland oats by parent races and hybrids of *Ustilago avenae*, depicting the mode of inheritance of virulence and avirulence.

| Parents and hybrids | Infection on Gothland and factors for virulence[a] | | | | Ratio[b] |
|---|---|---|---|---|---|
| | Dicaryon-1 | Dicaryon-2 | Dicaryon-3 | Dicaryon-4 | |
| Race 2 | 0 (GG) | 0 (GG) | 0 (GG) | 0 (GG) | 4A:0V |
| Race 7 | 70 (gg) | 78 (gg) | 80 (gg) | 70 (gg) | 0A:4V |
| Race 7b | 82 (gg) | 93 (gg) | 91 (gg) | 86 (gg) | 0A:4V |
| $F_1$ (2×7)[c] | 0 (Gg) | 0 (Gg) | 0 (Gg) | 0 (Gg) | 8A:0V |
| $F_1$ (2×7b)[c] | 0 (Gg) | 0 (Gg) | 0 (Gg) | 0 (Gg) | 8A:0V |
| $F_2$ (2×7) | 0 (Gg) | 0 (GG) | 100 (gg) | 0 (Gg) | 3A:1V |
| $F_2$ (2×7b) | 64 (gg) | 0 (Gg) | 0 (Gg) | 0 (GG) | 3A:1V |

[a] In Tables 2–4, inclusive, numbers are percentages of smutted oat panicles indicating actual virulence. In this table the letters in parentheses are virulent (gg) and avirulent (GG, Gg) gene symbols.

[b] In Tables 2–4, inclusive, A and V in this column denote avirulent and virulent phenotypes, respectively. Selfed spores give rise to 4 dicaryons; crosses between spores produce 8 hybrid $F_1$ dicaryons.

[c] In Tables 2–4, inclusive, infection percentages given for $F_1$ are averages for 8 hybrid dicaryons.

virulent segregants among $F_2$ progeny is determined by calculating the respective infection percentages of the 4 dicaryons on a given host variety. These reactions are usually clearly expressed as "3 avirulent to 1 virulent" phenotypes in the $F_2$. Hence, the character shown by the heterozygote (avirulence) is dominant. The 3:1 ratios also indicate simple recessive inheritance of the character (virulence) studied.

*Virulence genes (gg) for Gothland.*—A study of the reactions of selfed $F_2$ lines derived from hybrid $F_1$ spores reveals segregation for virulence among the constituent race progenies (Table 2). The fact that parent races A-7 and A-7b are separable from A-2 by distinct pathogenic reactions on Gothland (Table 1) reflects genetic control of virulence. The evidence for dominance of avirulence and for single-gene control of virulence on Gothland oats is presented in Table 2. Dominance of avirulence is shown by the uniform avirulence in the $F_1$ of the 16 hybrid dicaryons resulting from two crosses of avirulent × virulent races (2×7, 2×7b). Single gene control of virulence is indicated by the infection percentages (reactions) and the genetic analysis (genotypes) of one segregating $F_2$ dicaryon from each race hybrid on Gothland oats. The homozygous recessive segregants (gg) are virulent, whereas the heterozygotes (Gg) and homozygous dominants (GG) are avirulent. The $F_2$ populations, each consisting of 4 dicaryons, segregate clearly in a ratio of 3 avirulent to 1 virulent phenotypes. Identical 3:1 ratios were obtained from an analysis of 8 $F_1$ spores of these two hybrids. These data confirm the dominance of avirulence and the simple recessive control of virulence in races of *U. avenae* on Gothland oats.

*Virulence genes (mm) for Monarch.*—Dominance of avirulence and monogenic control of virulence are also indicated by the pathogenic patterns of race hybrids on Monarch oats (Table 3). The $F_1$ populations derived from crosses of avirulent × virulent races (5×6, 5×7, 7b×7) resulted in consistent expressions of avirulence among 24 individual hybrid dicaryons. These reactions indicate dominance of avirulence in the $F_1$. The 3 $F_2$ populations derived by selfing one heterozygous (Mm) spore of each cross produced in each instance a uniform expression of 3 avirulent to 1 virulent phenotypes on Monarch oats. Identical ratios were obtained from an analysis of 9 $F_1$ spores of these three hybrids. These 9 $F_2$ ratios indicate monogenic recessive control of virulence. Thus, the data in Table 3 show that, on Monarch, avirulence is dominant (MM,Mm), virulence is recessive (mm), and a single factor pair governs expression of this character in these *U. avenae* races.

*Virulence genes (cc) for Camas.*—Dominance of

**Table 3.**—The infection percentages, accompanied by relevant gene symbols, produced on Monarch oats by parent races and hybrids of *Ustilago avenae*, depicting the mode of inheritance of virulence and avirulence.

| Parents and hybrids | Infection on Monarch and factors for virulence[a] | | | | Ratio |
|---|---|---|---|---|---|
| | Dicaryon-1 | Dicaryon-2 | Dicaryon-3 | Dicaryon-4 | |
| Race 5 | 0 (MM) | 0 (MM) | 3 (MM) | 0 (MM) | 4A:0V |
| Race 6 | 0 (MM) | 0 (MM) | 0 (MM) | 0 (MM) | 4A:0V |
| Race 7 | 50 (mm) | 56 (mm) | 65 (mm) | 73 (mm) | 0A:4V |
| Race 7b | 95 (mm) | 96 (mm) | 94 (mm) | 98 (mm) | 0A:4V |
| $F_1$ (5×6) | 5 (Mm) | 5 (Mm) | 5 (Mm) | 5 (Mm) | 8A:0V |
| $F_1$ (5×7) | 5 (Mm) | 5 (Mm) | 5 (Mm) | 5 (Mm) | 8A:0V |
| $F_1$ (7b×7) | 0 (Mm) | 0 (Mm) | 0 (Mm) | 0 (Mm) | 8A:0V |
| $F_2$ (5×6) | 8 (Mm) | 0 (MM) | 93 (mm) | 8 (Mm) | 3A:1V |
| $F_2$ (5×7) | 0 (MM) | 9 (Mm) | 8 (Mm) | 98 (mm) | 3A:1V |
| $F_2$ (7b×7) | 0 (Mm) | 0 (MM) | 100 (mm) | 0 (Mm) | 3A:1V |

[a] See Table 2 footnotes. In this table the letters in parentheses are virulent (mm) and avirulent (MM, Mm) gene symbols.

**Table 4.**—The infection percentages, accompanied by relevant gene symbols, produced on Camas oats by parent races and hybrids of *Ustilago avenae*, depicting mode of inheritance of virulance and avirulence.

| Parents and hybrids | Infection on Camas and factors for virulence[a] | | | | Ratio |
|---|---|---|---|---|---|
| | Dicaryon-1 | Dicaryon-2 | Dicaryon-3 | Dicaryon-4 | |
| Race 5 | 0 (CC) | 0 (CC) | 0 (CC) | 0 (CC) | 4A:0V |
| Race 6 | 0 (CC) | 0 (CC) | 0 (CC) | 0 (CC) | 4A:0V |
| Race 7 | 67 (cc) | 66 (cc) | 74 (cc) | 81 (cc) | 0A:4V |
| Race 7b | 35 (cc) | 48 (cc) | 64 (cc) | 24 (cc) | 0A:4V |
| $F_1$ (5×7) | 1 (Cc) | 1 (Cc) | 1 (Cc) | 1 (Cc) | 8A:0V |
| $F_1$ (6×7) | 0 (Cc) | 0 (Cc) | 1 (Cc) | 0 (Cc) | 8A:0V |
| $F_1$ (6×7b) | 0 (Cc) | 0 (Cc) | 0 (Cc) | 0 (Cc) | 8A:0V |
| $F_2$ (5×7) | 100 (cc) | 0 (Cc) | 3 (Cc) | 2 (CC) | 3A:1V |
| $F_2$ (6×7) | 2 (Cc) | 0 (CC) | 74 (cc) | 4 (Cc) | 3A:1V |
| $F_2$ (6×7b) | 0 (Cc) | 0 (CC) | 86 (cc) | 0 (Cc) | 3A:1V |

[a] See Table 2 footnotes. In this table the letters in parentheses are virulent (cc) and avirulent (CC, Cc) gene symbols.

avirulence was also evident in the pathogenic patterns displayed on Camas oats by 24 hybrid $F_1$ lines representing avirulent × virulent race crosses (5×7, 6×7, 6×7b; Table 4). The data from 3 representative populations (Table 4) clearly display segregation of 3 avirulent to 1 virulent phenotypic reactions on Camas. Ten additional $F_2$ populations from these 3 hybrids also displayed 3:1 ratios. Thus, these 13 populations confirm that avirulence is dominant (CC, Cc) and indicate that virulence is inherited as a simple recessive character (cc) with respect to pathogenicity on Camas oats.

**Discussion.**—The existence of discrete pathogenic races in *Ustilago avenae* reflects genetic control of virulence, a fact well established by previous research (2, 3, 4, 7). The heritable nature of virulence led Halisky (2) to designate gene symbols (*mm*, *cc*) for factors governing its expression on specific host varieties. The extension of symbol designations for virulence genes (*aa*, *gg*) in the present study clarifies the relation between differences in virulence among smut races and their respective genotypes. Thus, as shown in Table 1, the existence of certain well defined races (phenotypes) of *U. avenae* is predicated on a multiplicity of genes for virulence (*aa gg mm cc*) arranged in various combinations (genotypes) within the parasitic dicaryons. The establishment of the concept that avirulence is dominant (Table 1) further clarifies the mode of inheritance of these factors, and the delimitation, identity, and designation of single recessive gene pairs governing virulence on specific host varieties (Tables 2, 3, 4) indicates monogenic control of this character.

The existence of multiple genes for virulence in *U. avenae* also suggests a possible mechanism for the origin of new races of the pathogen. Thus, a meiotic reshuffling of existing genes (*aa gg mm cc*) into new combinations (genotypes) might lead to the evolution of new races (phenotypes), which was demonstrated in a study of an avirulent × virulent race cross segregating for virulence at two loci (Table 5). The cross [A-5 (aaggMMCC) × A-7 (aaggmmcc)] involves heterozygosity at loci "M" and "C," respectively. Selfing of the $F_1$ heterozygotes (aaggMmCc) produced $F_2$ dicaryons that segregated for virulence at both loci. The resulting $F_2$ population (Table 5) portrays the origin of new genetic combinations (aaggMMcc; aaggmmCC) and, consequently, phenotypic segregants (A-6; A-7b) unlike either parent (A-5, A-7). The experimental production of a previously unknown genotype (aaggMMcc) through hybridization suggests a possible mechanism for the evolution of new pheno-

**Table 5.**—The pathogenic patterns and theoretical gene complexes of races A-5 and A-7, an $F^1$ hybrid, and $F^2$ progeny showing genotypically the possible origin of race A7b of *Ustilago avenae* through interracial hybridization.

| Races, hybrid, and segregants | Host variety, dicaryon reaction, and genes for virulence[a] | | | | Genetic analysis | |
|---|---|---|---|---|---|---|
| | Anthony | Gothland | Monarch | Camas | Genotype | Phenotype |
| Race 5 | 88 (aa) | 82 (gg) | 0 (MM) | 0 (CC) | aaggMMCC | A-5 |
| Race 7 | 78 (aa) | 80 (gg) | 91 (mm) | 53 (cc) | aaggmmcc | A-7 |
| 5×7 $F_1$ | 99 (aa) | 95 (gg) | 5 (Mm) | 2 (Cc) | aaggMmCc | A-5 |
| $F_2$[b] | | | | | | |
| 1×2 | 89 (aa) | 94 (gg) | 0 (MM) | 93 (cc) | aaggMMcc | A-7b |
| 1×4 | 88 (aa) | 95 (gg) | 9 (Mm) | 0 (Cc) | aaggMmCc | A-5 |
| 3×2 | 96 (aa) | 97 (gg) | 12 (Mm) | 0 (Cc) | aaggMmCc | A-5 |
| 3×4 | 93 (aa) | 95 (gg) | 99 (mm) | 0 (CC) | aaggmmCC | A-6 |

[a] Virulence indicated by actual infection percentages obtained by Halisky (2). In this table the letters in parentheses denote virulent genes on Anthony (aa), Kothland (gg), Monarch (mm), and Camas (cc), and avirulent combinations on Monarch (MM, Mm).

[b] Four segregant populations from one hybrid spore, obtained by crossing the four monosporidial lines in all compatible combinations.

types, such as A-7b, and clarifies the role of virulence genes in relation to the race dynamics of the species.

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# DIFFERENCE IN THE BEHAVIOR OF THE NICOTIANA LONGIFLORA WILDFIRE RESISTANCE LOCUS IN TOBACCO VARIETIES BURLEY 21 AND KY 61

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SUMMARY

The *Nicotiana longiflora* factor for wildfire resistance is present in *N. tabacum* vars. Burley 21 and Ky 61. Burley 21 was more resistant to wildfire than Ky 61 even though the same species was used as a source of resistance. In comparisons between $F_1$ and $F_2$ progenies of resistant × susceptible, Burley 21 transmitted resistance as expected for a single dominant factor whereas Ky 61 progenies contained more susceptible plants than expected. Gamete selection through the pollen or an embryonic lethal could have influenced progeny ratios when Ky 61 was involved. The data suggest that the wildfire locus was translocated to different chromosomes in Burley 21 and Ky 61.

Cultivated tobacco, *Nicotiana tabacum* L. (n = 24), was susceptible to the bacterium *Pseudomonas tabaci* (Wolf & Foster) Stevens, which causes the wildfire disease of tobacco. Investigations of related species in the genus *Nicotiana* have shown resistance to the organism in several *Nicotiana* species (1, 4, 6). *Nicotiana longiflora* (n = 10) was used in transferring resistance to cultivated tobacco. Respective products of the U.S.D.A. and the Kentucky Agricultural Experiment Station were the resistant varieties Burley 21 and Ky 61.

In 1956 it was noted that, in crosses to susceptible tobacco, Burley 21 yielded a uniformly resistant $F_1$ whereas Ky 61 did not. Investigations were undertaken to determine the reasons for the difference. The breeding lines leading to these varieties were derived from different source materials.

**Methods.**—A single plant was selected from a progeny of Burley 21 that was uniformly resistant. Similar attempts failed to obtain a uniformly resistant Ky 61 line. Therefore, a single-plant line, Ky 61-5, was selected that represented the most resistant single-plant line found in Ky 61 (Table 1). Ky 16 was used as the susceptible parent in all crosses.

The bacterium was carried *in vivo* (Ky 16). Transfers were made with small (⅛×¼-in.) sections of diseased tissue cultured 24 hours in beef broth at room temperature. Four isolates from field cultures were mixed and diluted 1:1000 for field inoculations and 1:2000 for greenhouse inoculations.

Field inoculations were made with a boom-type spray rig so designed that inoculum could be directed to the lower surfaces of leaves, since inoculum enters a leaf through stomata (2). Five-gallon 70° T-jet nozzles at 150 lb pressure per in. gave a droplet size adequate to cause incipient water-soaking.

Greenhouse inoculations were made with a deVilbiss paint spray gun adjusted to deliver 10 ml per 10 seconds at 15 lb pressure per sq in. The pressure was then lowered to the least pressure that would cause water-soaking.

**Results.**—Ky 61 appeared to be heterozygous when selfed progenies were scored; the testcrosses, however, yielded more numerous resistant plants than the expected 1:1 for a single dominant factor (Table 1). This characteristic was common throughout the study in work with Ky 61 testcross progenies. Progenies of crosses, testcrosses, and selfs were grown in the field, inoculated, and scored in 1958 (Tables 2, 3, 4).

**Table 1.**—Reaction of self and testcross progenies of Ky 61 plants after inoculation by *Pseudomonas tabaci*.[a]

| Plant | Self | | Testcross | |
|---|---|---|---|---|
| | Res. | Sus. | Res. | Sus. |
| Ky 61-1 | 24 | 8 | 19 | 13 |
| Ky 61-2 | 27 | 5 | 23 | 9 |
| Ky 61-3 | 25 | 7 | 19 | 13 |
| Ky 61-4 | 24 | 8 | 23 | 9 |
| Ky 61-5 | 27 | 5 | 26 | 6 |
| Ky 61-6 | 25 | 7 | 22 | 10 |
| Ky 61-7 | 7 | 25 | 6 | 26 |
| Ky 61-8 | 24 | 8 | 24 | 8 |
| Ky 61-9 | 24 | 8 | 17 | 15 |

[a] Greenhouse 1957.

**Table 2.**—Results and $\chi^2$ analysis after inoculation by *Pseudomonas tabaci*.[a]

| Parentage | Number of plants | | Expected ratio | $\chi^2$ | P |
|---|---|---|---|---|---|
| | Res. | Sus. | | | |
| Burley 21 | 80 | 0 | | | |
| $F_1$ Bur 21 × Ky 16 | 78 | 1 | | | |
| $F_2$ Bur 21 × Ky 16 | 98 | 31 | 3:1 | .06 | .8 |
| TC(Bur 21 × Ky 16) × Ky 16 | 42 | 38 | 1:1 | .2 | .7 |
| Ky 61-5 | 58 | 24 | | | |
| $F_1$ Ky 61-5 × Ky 16 | 56 | 25 | | | |
| TC(Ky 61-5 × Ky 16)-1 × Ky 16 | 43 | 31 | 1:1 | 1.94 | .17 |
| $F_1$ Ky 61-5 × Bur 21 | 77 | 0 | | | |
| TC(Ky 61-5 × Bur 21)-1 × Ky 16 | 48 | 16 | 3:1 | 0.0 | 1.0 |
| Ky 16 | 0 | 80 | | | |

[a] Field data 1958.

The Burley 21 plant progenies gave the expected ratios for a single dominant factor as previously reported by Clayton (1). Three of the $F_2$ Ky 61-5 × Ky 16 progenies (plants 3, 4, and 6) were acceptable for the 3:1 goodness of fit, whereas the other individual $F_2$ progenies (plants 1, 2, and 5) indicated poor transmission of the Ky 61-5 locus even though the $F_1$ Ky 61-5 × Ky 16 was resistant and therefore carried the *N. longiflora* locus (Table 3). Three $F_2$ Ky 61-5 × Bur 21 progenies (plants 1, 3, and 4) were acceptable to the expected 15:1 ratio, whereas the 2 other $F_2$ progenies (plants 2 and 5) were not acceptable (Table 4). Testcrosses gave the expected ratios and were acceptable throughout the progeny test.

**Discussion.**—It should be noted that the *N. longiflora* locus does not confer complete dominance, since homozygous *N. tabacum* lines show more resistance than the heterozygotes (3).

The Burley 21 line was homozygous resistant and the progenies of crosses and testcrosses segregated in the expected manner. Throughout the study, resistant plants of Ky 61 were not as resistant as those of Burley 21. Since resistance is relative and both resistant Burley 21 and Ky 61 plants will show a susceptible reaction when concentrations of inoculum are high, slight differences between varieties in ease of water-soaking (5) could be a factor.

When susceptible Ky 61 plants were selfed and the progenies tested, they were uniformly susceptible, whereas resistant plants selfed produced progenies that always segregated.

Since Ky 61-5 resistance was transmitted through the female parent in all crosses and testcrosses involving Ky 61-5 and the per cent stainable pollen was normal, gamete selection through the pollen or an embryonic lethal could not be eliminated. However, the progeny ratio from Ky 61-5 selfed was skewed to the susceptible side whereas the TC (Ky 61-5 × Ky 16)-1 × Ky 16 gave the expected 1:1 ratio (Table 2). This point, suggesting male gamete selection or a homozygotic lethal, awaits further study.

If Ky 61-5 were assumed to be heterozygous, the gametes would carry the factor for either resistance or susceptibility. Then, in crosses with Burley 21, an $F_2$ ratio of 3:1 would be expected if the gamete carrying the susceptible factor effected fertilization. If a gamete carrying the resistant factor were involved, however, the $F_2$ progeny would be uniformly resistant if the parental factors were on the same chromosome, and 15:1 if not.

Three of the 5 $F_2$ Ky 61-5 × Burley 21 progenies were acceptable to the expected 15:1 and the TC(Ky 61-5 × Burley 21)-1 × Ky 16 fits the expected 3:1 ratio when different chromosome locations were assumed. The data more nearly fit the assumption that the *N. longiflora* factor is located on different chromosomes in the two varieties rather than on the same

**Table 3.**—Results and $\chi^2$ analysis (3:1) of $F_2$ Ky 61-5 × Ky 16 progenies after inoculation with *Pseudomonas tabaci*.

| $F_2$ Ky 61-5 × Ky 16 | | Number of plants | | $\chi^2$ | P |
|---|---|---|---|---|---|
| | | Res. | Sus. | | |
| Plant 1 | | 52 | 27 | 3.55 | .06 |
| Plant 2 | | 50 | 30 | 6.67 | .01 |
| Plant 3 | | 60 | 18 | .16 | .70 |
| Plant 4 | | 54 | 23 | .97 | .30 |
| Plant 5 | | 53 | 27 | 3.27 | .07 |
| Plant 6 | | 47 | 21 | 1.25 | .25 |
| | D.F. | 316 | 146 | | |
| Total | 6 | | | 15.87 | |
| Pooled | 1 | | | 10.73 | |
| Heterogeneity | 5 | | | 5.14 | .275 |

**Table 4.**—Results and $\chi^2$ analysis (15:1) of $F_2$ Ky 61-5 × Bur 21 progenies after inoculation with *Pseudomonas tabaci*.

| $F_2$ Ky 61-5 × Bur 21 | | Number of plants | | $\chi^2$ | P |
|---|---|---|---|---|---|
| | | Res. | Sus. | | |
| Plant 1 | | 73 | 6 | .26 | .7 |
| Plant 2 | | 68 | 11 | 8.07 | .004 |
| Plant 3 | | 72 | 6 | .32 | .6 |
| Plant 4 | | 77 | 4 | .26 | .7 |
| Plant 5 | | 64 | 15 | 22.20 | .0001 |
| | | 354 | 42 | | |
| Total | 5 | | | 31.11 | |
| Pooled | 1 | | | 13.32 | |
| Heterogeneity | 4 | | | 17.79 | .002 |

chromosome.—Kentucky Agricultural Experiment Station, Lexington.

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# STIMULATION OF BROOMRAPE SEED GERMINATION

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SUMMARY

Gibberellic acid (1–20 ppm) in agar stimulated the germination of seeds of *Orobanche ramosa* and *O. ludoviciana* var. *cooperi*. *O. ramosa* seeds did germinate on yeast-extract agar, nutrient agar, or agar containing vitamins B6 or niacinamide (100 ppm). *O. ludoviciana* seeds germinated on water agar. Germination of both increased with the concentration of gibberellic acid in the agar. In nature, gibberellins may be secreted in small amounts into the rhizosphere of young plants, and thus may play a part in the germination of broomrape seeds. After seed germination started on the agar surface (in 2-3 weeks), the radicle grew to 2–3 mm long (*O. ludoviciana*) or 1.5 mm long (*O. ramosa*). Final length was reached in about a day. The gibberellic acid level did not affect radicle length.

For centuries broomrape, *Orobanche* sp., has been recognized as a destructive parasite of higher plants. Several species have been known in California for at least 3 decades, but control received little attention until their recent spread. Tomato and other crop plants are affected.

The seeds of broomrape are very small, live in the soil indefinitely, and require a close association with the roots of susceptible host plants in order to establish their parasitic habit. Two general approaches to control have therefore been made: 1) eradication of the seed by chemical treatment of the soil (17), and 2) induction of seed germination in the absence of a host. The second is discussed herein.

Though some held to the theory of spontaneous generation of Orobanche (9), Vaucher (14, 15) first demonstrated (in 1823) that its seeds normally germinate only in the immediate vicinity of a host root. Wight (17) considered it self-evident that root secretions stimulated germination of broomrape seed. Chabrolin, in a series of papers, the last in 1939 (7), described a dry residue, obtained from aqueous extracts of roots, that, redissolved in water, caused germination of seeds of *Orobanche speciosa* DC (*O. crenata* Forsk.).

Barcinsky (1, 2) reported that cellular contents of sunflower roots, bark, stem, tissue, and leaves contained a substance that stimulated germination of *O. cumana* Wallr. seed. This stimulant was shown to withstand boiling for ½ hour and to be present in certain nonhosts such as Jerusalem artichoke, but absent from wheat, barley, and maize. He further reported complete control of *O. cumana* by incorporating 40–65 g of dried sunflower meal per kg of infested soil (3).

Brown et al (4, 5, 5) extracted, purified, and characterized the stimulant for *Orobanche minor* Sm. seeds from charcoal eluates that had been allowed to adsorb the stimulant from root secretions of flax seedlings. They recognized 2 phases in the total host-parasite relationship of the broomrapes and their respective hosts: 1) stimulation of seed germination by the host root, and 2) seedling attachment to the root of the host. They observed that flax plants, which are not parasitized by *O. minor* in the field, nevertheless produce sizable quantities of a seed-germination stimulant, and are more active in inducing the seed germination than are legumes, the normal host for this species of the parasite. Apparently a great variety of plant roots ordinarily secrete the germination stimulant into their rhizospheres. Flax was the most effective of the species tested by Brown's group.

Davies (8) attempted to control *Orobanche minor* Sm. in a very heavily infested red clover field in Wales by growing crops of flax prior to the clover. Broomrape plant counts before and after the flax crop indicated a mean decrease of 21% for the 4 blocks grown.

Izard and Hitier (11, 12) reported the vitamins containing a pyridine nucleus, pyridoxal and niacinamide, stimulated germination of *Orobanche ramosa* L. seed. The present work, however, does not confirm their results, with the lots of *Orobanche ramosa* tested. Nor was Brown's group able to show stimulation of *Orobanche minor* seed germination with these or other vitamins tested (2). Guidice (10) reported 15% germination of the seed of *O. speciosa* DC after a 24-hour treatment in concentrated ammonia.

The compound or compounds isolated by Brown et al were described as stimulatory at dilutions of $10^{-3}$–$10^{-6}$. Chemical and spectrophotometric analyses showed evidence of a potential acid group, of a lactone or ester group, of a methyl group, of a hydroxyl group, and of a double bond. No sulfur, nitrogen, phosphorus,

**Table 1.**—Germination percentages of various lots of *Orobanche* seeds at concentrations of gibberellic acid from 0 to 100 ppm.

| Species | Gibberellic acid (ppm) | No. seeds counted | Incubation time (days) | Germination (%) |
|---|---|---|---|---|
| *O. ramosa* —lot 1 | 0 | 161 | 18 | 0 |
| | 1 | 55 | | 0 |
| | 2 | 106 | | 1.9 |
| | 20 | 213 | | 7.5 |
| | 100 | 148 | | 17.0 |
| *O. ramosa* —lot 2 | 0 | 5573 | 21 | 0.21 |
| | 1 | 5133 | | 0.52 |
| | 20 | 5475 | | 2.24 |
| *O. ramosa* —lot 3 | 0 | 2972 | 14 | 0 |
| | | | 35 | 0 |
| | 1 | 2026 | 14 | 0.28 |
| | 5 | 2749 | 14 | 1.44 |
| | | | 35 | 2.40 |
| *O. ludoviciana* var. *cooperi* | 0 | 1789 | 21 | 20.5 |
| | 1 | 1707 | | 34.5 |
| | 20 | 1667 | | 47.3 |

or halogen was present. The carbon-hydrogen analysis revealed a carbon content of 59.5–62%, hydrogen 7.6–8.7%. The use of n-butanol–acetic-acid–water paper-strip chromatograms in purifying the crude extracts revealed biological activity in the fractions traveling close to the solvent front with RF values greater than 0.8 and distinct from the extract fractions containing carbohydrates, which have RF values less than 0.5 on this type of chromatogram. Since the active region is wide, there is a strong possibility that several closely related substances were concerned in stimulation rather than one substance.

The work of Brown's group seems to implicate gibberellin-like substances as stimulatory substances for broomrape seed germination. To test this implication, gibberellic acid was investigated as a stimulant of germination for 2 species of broomrape.

**Materials and methods.**—The seeds used were harvested from infestations in tomato fields in 2 areas in California—*O. ramosa* from the Centerville area, Alameda county; and *O. ludoviciana* var. *cooperi* (Gray) Beck, a native broomrape, from the Mecca-Indio area, Riverside county. The seeds were washed and suspended in a solution of Tween 80 in order to wet them. They were then surface-sterilized in 10% Clorox for 2 minutes, and washed several times to remove the Clorox. One ml of each suspension was pipetted onto the surface of a poured Petri plate containing 2% washed-sterilized agar with filter-sterilized gibberellic acid incorporated in it at various levels up to 100 ppm. Three lots of the *O. ramosa* and one of the *O. ludoviciana* seed were tested.

Incubation was at room temperature in the dark. The number of seeds germinating was determined with the dissecting microscope. The results were read at 18 days in one test, 14 days and again at 35 days in another test, and at 21 days in a third test. It was observed that the seeds completed germination within 3 weeks.

Surface sterilization with Clorox was found necessary for the seed lots used. Otherwise, fungi grew profusely over the seed and the surface of the agar plate, causing the seed to rot before the normal time of germination.

**Results.**—*O. ludoviciana* seeds germinated spontaneously without gibberellic acid, but germination was significantly increased by gibberellic acid (Table 1). The *O. ramosa* seeds germinated much less abundantly, but showed a definite stimulation with gibberellic acid even at very low levels. Germination tended to increase with increasing concentrations of gibberellic acid.

Niacinamide and pyridoxin at 100 ppm did not stimulate germination in either species of broomrape tested, nor did nutrient or yeast-extract agars. Viability in a lot of *O. ramosa* seed was estimated at 10–15% by repeated planting (ten) of tomatoes to pots of soil infested with known numbers of seed. Since *O. ramosa* is not native to California it is possible that lack of viability may be due to poor pollination. When seed was stored for 4 years and re-examined under a dissecting microscope, many appeared smaller, empty, and light in color. Since the germination per cent of the lot had not materially changed from the original count it is believed that these shrunken seeds had never been viable.

It was noted that the radicles of the germinated seeds of *O. ludoviciana* became about 2–3 mm long (Fig. 1), and progressed no further regardless of how long the plates were held. From the time of seed germination, growth of the radicle required about a day, although 2 weeks often elapsed before the dry seeds placed on the agar surface began to germinate. Though percentage of germination increased with increasing amounts of gibberellic acid, radicle length was not affected. A tiny knob is located at the tip of each radicle (Fig. 1). *O. ramosa* seeds, which are smaller (about 0.2 mm in diameter), produced radicles up to 1.5 mm long, with a less obvious knob.

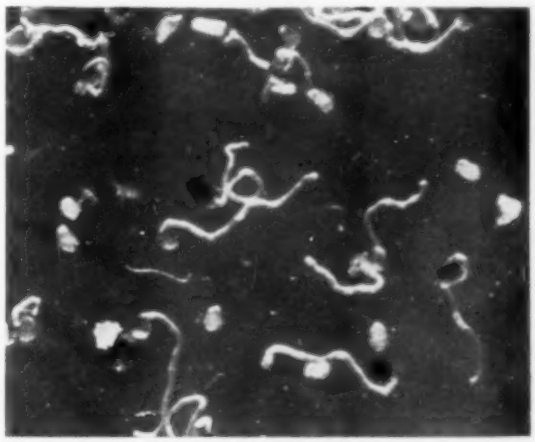

**Fig. 1.**—Seeds of *Orobanche ludoviciana* var. *cooperi* (Gray) Beck at 21 days of germination on water agar containing 20 ppm gibberellic acid. The radicles in this species reached a length of 2–3 mm and had a definite knob at the end. No branching or secondary rootlets were observed.

**Discussion.**—It seems reasonable to assume that plant gibberellins (13, 16) secreted into the rhizosphere by roots may contribute substantially to the stimulation of germination of Orobanche seeds. The tiny seedlings then may independently reach a length of a few mm, but if they are unable to penetrate a host plant they die. Thus seeds stimulated by flax would be destroyed. Even so, it is questionable whether trap crops could give efficient control. Orobanche plants ordinarily produce great quantities of seeds, which remain viable in the soil for many years. Relatively few could be eliminated with each planting of a trap crop if germination and death depend on proper depth, location within the rhizosphere of a rootlet actively secreting stimulant and in proper stage of development, and proper moisture content. Combining a suitable rotation sequence with a good fumigation program (18), however, might be more effective than either practice alone.—Department of Plant Pathology, University of California, Berkeley 4.

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## PHYTOPATHOLOGICAL NOTES

### COCHLIOBOLUS VICTORIAE, THE PERFECT STAGE OF HELMINTHOSPORIUM VICTORIAE

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In a continued study of the evolution of sexuality in fungi, certain wild-type isolates of *Helminthosporium victoriae* Meehan & Murphy, causal agent of a seedling blight and culm rot of oat varieties with Victoria parentage, were observed to be cross-compatible with certain isolates of *Cochliobolus carbonum* Nelson (*Helminthosporium carbonum* Ullstrup). When additional isolates of the 2 species were tested, some isolates of *H. victoriae* were compatible with 1 mating type of *C. carbonum* and others were cross-fertile with the other. The perfect stage of *H. victoriae* was then obtained by pairing these strains. The methods used in obtaining fertile perithecia of the fungus in paired cultures are reported herein. The morphology and taxonomy of the species are discussed, and evidence of its heterothallic nature is presented.

**Materials and methods.**—The fungi used were 22 conidial isolates from diseased oats from Mississippi, Florida, Iowa, Georgia, Texas, Louisiana, Wyoming, North Carolina, and Wisconsin. Also studied were 31 monoascosporic cultures isolated from perithecia produced in paired cultures.

Mature perithecia were obtained when small pieces of mycelium of compatible isolates were placed on opposite sides of a section of sterile corn leaf placed in the center of a Petri plate containing Sach's nutrient agar. The sections of corn leaf, about 1 × 1 in., were sterilized by autoclaving 1 hour. Fertile perithecia were also formed when isolates were paired on opposite sides of sterile barley grains, but their numbers were usually fewer. Numerous pairings on

opposite sides of small pieces of oat leaves failed, as did several matings of known compatible strains on agar media without leaf tissue or grains.

Mature perithecia developed most abundantly and consistently when paired cultures were grown on a medium of pH 4 and kept continually at 24°C. Additional studies indicated that light was not a factor in sexual reproduction. Under optimum conditions, immature perithecia appeared after 6-7 days and immature asci after 11–13 days. A small proportion of the asci contained mature ascospores after 15 days, and most of the functional asci contained mature spores after 22 days.

**Morphology and taxonomy.**—Mature perithecia are black and ellipsoidal to globose. They measure 225-430 $\mu$ in height and 210-370 $\mu$ in diameter. Long brown setae are produced over the upper third of the perithecium. Conidiophores and conidia frequently develop on the perithecium. A well-defined ostiolate beak, subconical to paraboloid and 30–170 $\mu$ long, is usually produced. A mass of hyaline cells frequently covers the apex of the beak.

The locule is filled with a mass of hyaline, filamentous pseudoparaphyses. Asci are produced among the pseudoparaphyses and arise from the base of the locule. The asci are cylindrical or clavate and straight or slightly curved, with a short stipe. Asci appear to be unitunicate, for there is no evidence of an extensible inner wall. They measure 98–207×20.4–38.7 $\mu$ (mean 137.5×26.8 $\mu$). Asci contain 1–8 ascospores coiled in a close helix. Ascospores are discharged by the splitting of the upper part of the ascus wall.

The ascospores are filiform or flagelliform, and somewhat tapered at the extremities. Mature ascospores are typically hyaline, with 5–9 septa, and measure 147–302×6.5–13.0 $\mu$ (mean 226.5×8.1 $\mu$). Following discharge, the ascospores are often surrounded by a thin mucous envelope. Germination is terminal or lateral.

The fungus belongs in the genus *Cochliobolus*, erected by Drechsler (1) to include species formerly considered in *Ophiobolus*, but differing in having filiform ascospores in helicoid arrangement and *Helminthosporium* conidial stages. Since *H. victoriae* is considered a valid conidial stage, no perfect stage has hitherto been associated with it. The fungus is described as a new species of *Cochliobolus*, as follows:

**Cochliobolus victoriae sp. nov.**—Ascomata nigra, ellipsoideo-globosa, ostiolata, 225–430 $\mu$ alta 210–370 $\mu$ in diametro, per quadrantem superiorem setis longis brunneis tecta, saepe conidiophora et conidia ferentia; rostrum ostiolarem definitum, subconicum, usque paraboloideum, 30–170 $\mu$ longum, ad apicem saepe massa cellularum hyalinarum tectum; loculus massa pseudoparaphysium hyalinarum filamentosarum et ascorum impletus; asci cylindrico-clavati, recti vel subcurvati, breve stipitati, adparenter unitunicati, 98–207×20.4–38.7 $\mu$ (mediani 137.5×26.8 $\mu$); parte superiori fissente et ascosporas ejiciente; ascosporae 1–8, in helice stricta circinnati, filiformes vel flagelliformes, ad apices attenuatae, hyalinae, 5–9-septatae, 147–302×6.5–13.0 $\mu$ (226.5×8.1 $\mu$), e tegumento tenui mucoso circumdatae, terminaliter vel lateraliter germinantes.

Type specimens have been deposited in the National Fungus Collections, Beltsville, Maryland, and in the Herbarium of the Royal Botanic Garden, Kew.

**Heterothallism.**—Studies with selfed and paired cultures of conidial and ascosporic isolates of *C. victoriae* have indicated that the species is heterothallic. Mature perithecia were produced only when monoconidial or monoascosporic isolates were grown in pairs. Single isolates produced only protoperithecia or none at all.

The finding of the perfect stage of *H. victoriae* and its production in culture will make possible a study of the genetic mechanisms controlling pathogenicity and toxin production. Furthermore, studies on the genes controlling sexual reproduction should present information on the relationship of *H. victoriae* and related species. — North Carolina State College, Raleigh.

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## DISTRIBUTION OF CERATOCYSTIS FAGACEARUM IN ROOTS OF WILT-INFECTED OAKS IN NORTH CAROLINA

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The assistance of Horace J. Duncan, Forestry Aid, Southeastern Forest Experiment Station, is gratefully acknowledged.

Root grafts between oaks may be important as avenues of infection for short-distance spread of the wilt fungus, *Ceratocystis fagacearum* (Bretz) Hunt (3). It is not yet clear, however, how much of the short-distance spread in the southern Appalachians represents root-graft transmission.

A relevant observation is that 78% of wilting oaks examined in Kentucky, North Carolina, and Tennessee were within 30 ft of wilt-killed trees, and 96% were within 50 ft (2). Thus most wilt spread near known infection centers is within the probable root zones of infected oaks.

In 1958 and 1959, the frequency of root grafts as natural infection courts in short-distance oak wilt spread was studied. All lateral roots of 42 wilting oaks within 30 ft of wilt-killed trees in western North Carolina were cultured from samples taken about 1 ft from the stem. A patch of bark was removed from the root, the exposed wood was swabbed with alcohol and flamed, and increment hammer cores were obtained aseptically from the exposed area. Usually 3 cores were taken only around the upper ½ circumference of each root because of difficulty in sampling the underside of the root in position. They were placed in a culture tube containing liquid Barnett's medium (1). When the oak wilt fungus was present in the cores

**Table 1.**—Distance from stem at which oak wilt fungus was isolated from roots.

| Distance (ft.) | No. roots yielding fungus | No. trees involved |
|---|---|---|
| 3 | 38 | 21 |
| 6 | 29 | 17 |
| 9 | 21 | 13 |
| 12 | 12 | 10 |
| 15 | 9 | 7 |
| 18 | 5 | 5 |
| 21 | 3 | 3 |
| 24 | 1 | 1 |

from a given root, it was readily identifiable in 7–10 days of incubation at 70–75°F. There was very little contamination by other fungi.

A total of 329 roots were sampled, of which 46 roots of 23 trees at 17 infection centers contained the wilt fungus near the stem. Where possible, each infected root was carefully uncovered either to its end or to a diameter of ¼–½ in. so that it could be examined for grafts. In addition, sections 6 in. long were removed at average intervals of 3 ft along the exposed roots, and the sections were cultured in the laboratory. Culturing was done by peeling the bark from each section, swabbing the exposed wood with alcohol, flaming it, and cutting chips out of it around the entire circumference for culturing on Barnett's agar. Examining cultures after 3–4 days of incubation at 70–75°F usually made it possible to identify *C. fagacearum* even when other fungi were present.

The 46 infected roots occurred on the following host species, with the indicated ranges of diameters at breast height: *Quercus coccinea*, 14 trees, 4–26 in.; *Q. falcata*, one 8-in. tree; *Q. rubra*, 3 trees, 6–8 in.; *Q. velutina*, 5 trees, 2–12 in. About 920 linear feet of roots were exposed, from which 325 root sections were removed. Laboratory culturing yielded the wilt fungus from 110 of these sections.

Three intertree root grafts were found. In one root graft the pathogen was isolated from both roots of the graft, thereby evidencing a pathway of infection. In this case, a currently wilting 17-in. scarlet oak was grafted to a root of an oak that had died of wilt the previous year. Both grafted roots were 1½ in. thick at the union.

In one of the other two intertree root grafts the wilt fungus was not isolated from the root of the current wilt tree near the point of union or from the dead root of the infected stump. In the other case the root of the wilting tree was grafted to a living tree that showed no symptoms of wilt, and the wilt fungus was not isolated from the healthy tree's root although it was isolated from the wilting tree's root near the graft. Self-grafts were present in the roots of 5 trees.

Distribution of the wilt fungus in the sampled roots is of interest (Table 1). *C. fagacearum* was isolated from 21 roots of 13 trees at least 9 ft from the stem. As mentioned, each of the sampled trees showed current wilt symptoms. In 1958 the test trees were cut during the period June 19 to July 8, and root sampling was completed by September 24. In 1959 the trees generally were not cut before root sampling, which was finished by August 13.

Thus, during both summers the wilt fungus was already present in many roots of wilting trees at an appreciable distance from the stem. In one root it was isolated 24 ft from the stem. The distribution of *C. fagacearum* within individual roots varied considerably. For example, 20 roots cultured positive at 3 or more sample points. The positive sample points were consecutive in 9 roots, but there were negative sample points between positive ones in 11 roots. One root was traced for 34 ft and sampled at 11 points 3 ft apart. It yielded the fungus only at the 18-foot sample point.

Root grafts were shown to be infection pathways in only 1 of the 23 oaks examined, although all these trees were within the root zones of wilt-killed trees. There is the possibility that some infected roots were missed during the initial sampling. For the infected roots located, however, the presence of grafts and distribution of the wilt fungus were determined reliably.

The presence of *C. fagacearum* in many ungrafted roots at appreciable distances from the stem could be explained by movement of the fungus into the roots from the stem or by some other means besides grafts. It was noted several times that roots of diseased and healthy oaks were in contact though not grafted; more often they were only a few inches apart. Thus, if soil vectors of the wilt fungus are involved in short-distance spread, the fungus need be transported only a few inches or less. Neither free growth of the fungus through soil nor soil vectors have yet been demonstrated, however.—Southeastern Forest Experiment Station, U. S. Department of Agriculture, Forest Service, Asheville, North Carolina.

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